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REPORT ON THE SIPUNCULOIDEA, ECHIUROIDEA AND
PRIAPULOIDEA COLLECTED BY THE SÔYÔ-MARU
EXPEDITION OF 1922-1930

By

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(With Pl. I and 31 text-figures)

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The Sipunculoidea, Echiuroidea and Priapulidea dealt with in this report were collected from the adjacent seas of Japan by the surveying-ship, "Sôyô-maru" of the Imperial Fisheries Institute in Tôkyô, Japan.

The species on which I have recorded here, are thirteen in number; of which eleven belong to Sipunculoidea, one to Echiuroidea and the remaining one to Priapulidea.

Of these forms, two are those uncertainly determined and four are identical with species or varieties previously known, while the remaining seven seem to be new to science.

The most remarkable fact regarding the collection is that there are found some specimens of Priapulidea. The occurrence of Priapulidea in Japanese water is reported for the first time.

The following is the list of species and varieties:

Sipunculoidea.

- 1) *Sipunculus nudus* LINNAEUS.
- 2) *Siphonosoma* sp.
- 3) *Phascolosoma vulgare* var. *tropicum* SLUITER.
- 4) *Phascolosoma margaritaceum* var. *antarcticum* MICHAELSEN.
- 5) *Phascolosoma appendiculatum*, n. sp.
- 6) *Phascolosoma glossipapillosum*, n. sp.
- 7) *Phascolosoma hyugense*, n. sp.
- 8) *Phascolosoma noto*, n. sp.
- 9) *Phascolosoma signum*, n. sp.
- 10) *Phascolosoma soyo*, n. sp.
- 11) *Dendrostoma ellipticum*, n. sp.

Echiuroidea.

- 12) *Thalassema* sp. (?)

Priapulidea.

- 13) *Priapulius bicaudatus* DANIELSSEN.

1. *Sipunculus nudus* LINNAEUS.

(Pl. I, Fig. 1).

Sipunculus nudus, LINNAEUS, 1766, Syst Nat 12 edit, p 1078; W. KEFERSTEIN, 1860, p. 1; 1865, pp. 418-419, 1867, pp 44-45; W BAIRD, 1868, p. 77; J. ANDREAE, 1881, pp 477-481, 1882, pp. 20-258, Pls XII-XIII, E SELENKA, 1883-1884, p 92, 1885, p. 22, H WARD, 1891, pp. 113-182, Pls I-III; A. SHIPLEY, 1893, p. 327; 1899, p. 158, W FISCHER, 1895, p 9; 1914, pp. 1 28, 1922, p. 5, Pl XXVI, Figs. 5-6, METALNIKOFF, 1903, pp 297-371; I. IKEDA, 1904, p. 31, 1905, p. 169; R. SOUTHERN, 1913, pp 1-46; J. GEROULD, 1913, p. 428; R. CHAMBERLIN, 1920, p. 30, L CUÉNOT, p 14; 1927, p. 249; A. TEN BROEKE, 1925, p. 2; H SATO, 1930, pp. 2-5, Pl I, Fig. 1, Text-fig 1.

Spec. No. A. 196; Station 271; N. Lat. 33° 19' 30'', E. Long. 134° 12' 15''; Depth, 421 m; Date, July 19, 1927; Coll. KONISHI and YAMASHITA.

Spec. No. A. 417; Station 297; N. Lat. 30° 57' 30'', E. Long. 131° 23' 00''; Depth, 516 m, Date, July 11, 1928; Coll. KAMIYA and MORIMOTO.

This extremely common species is represented in the collection by three specimens. The first specimen (Spec. No. A. 196; Pl. I, Fig. 1) was secured at a depth of 421 meters off Muroto-zaki, Shikoku; and the remaining two (Spec. No. A. 417) were obtained off Sata-misaki, Kyûshû.

The first specimen (Fig. 1) is smaller in size than those previously obtained from the other localities, and measures about 65 mm in length and 7 mm in width. The colour of the skin is light dirty purple when the animal is preserved in alcohol. The number of the longitudinal muscle-bands is 28-30. The intestinal convolution consists of about 10 double spirals. The rectal diverticulum is absent.

The other two specimens are smaller than the first, and the skin is thin being rather translucent.

Localities. Muroto-zaki, Shikoku; Sata-misaki, Kyûshû.

Remarks. Judging from these characteristics mentioned above, namely, 1) the smaller size of the body, 2) a small number of intestinal spirals, 3) the absence of rectal diverticulum and 4) the translucence of the body-wall, it seems to me that all these specimens represent the young forms of *Sipunculus nudus*.

2. *Siphonosoma* sp.

Spec. No. N. 66; Station 242; N. Lat. 35° 15' 45'', E. Long. 139° 27' 30''; Depth, 101 m; Date, Nov. 7, 1927; Coll. KAMIYA and MORIMOTO.

The collection contains a single extremely imperfect specimen, the posterior half of the body being torn off.

The skin is thin, and shows a grayish tint. Hooks are absent. The circular muscle layer forms a continuous sheet. The longitudinal muscle layer of the body-wall is divided into about 17-19 separate bands. Two pairs of the retractor muscles are found inside of the body-wall arising from the same level. The ventral pair arises from the 1st-2nd longitudinal muscle-bands, while the dorsal pair arises from the 5th-7th. Two segmental organs are present. They are long tubes of a reddish-yellow colour, and are free from the body-wall except for their most anterior part which is attached. The external apertures of these organs lie between 2nd and 3rd longitudinal muscle-bands at the level far distant anteriorly from the anus.

Locality. Sagami Sea.

Remarks. Judging from these features above mentioned it seems to be quite certain that this form is one of the members of the genus *Siphonosoma* and is closely allied to *Siphonosoma cumanense* (KEFERSTEIN). But owing to the imperfection of the specimen it is impossible to settle the specific name.

3. *Phascolosoma vulgare* var. *tropicum* SLUITER.

(Pl. I, Fig. 2, Text-figs 1-4)

Phascolosoma vulgare var. *tropicum*, SLUITER, 1902, pp. 33-34.

Spec. No. S. 2; Station 61; N. Lat. 40° 03' 24'', E. Long. 142° 11' 32''; Depth, 170 m; Date, July 21, 1926; Coll. MARUKAWA and YOSHIDA.

A single specimen (Pl. I, Fig. 2) was obtained at a depth of 170 meters off Fudai, a village on the Pacific coast of North Japan.

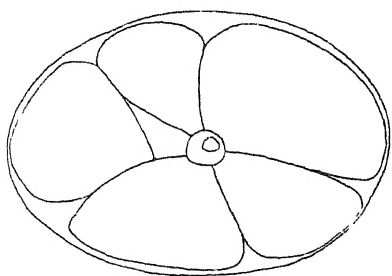
The trunk measures about 35 mm in length and 6 mm in thickness. The introvert is about two-thirds of the trunk-length.

The body-wall is thin, pale gray in colour and appears nearly smooth to the naked eye; while both in the anterior and the posterior regions of the trunk, the wall is rather thick, being yellowish-brown in colour and appears rough to the naked eye.

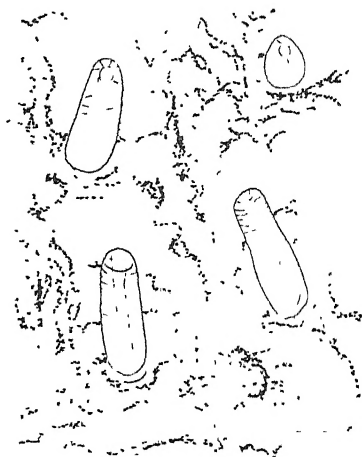
The papillae in the middle region of the trunk-surface are flat and elliptical in the surface view, measuring about 0.09 mm in major axis and about 0.065 mm in minor axis (Text-fig. 1). At the introvert-basis and at the posterior end of the body, the papillae are extremely tall and are cylindrical in form, measuring up to 0.13 mm in height (Text-fig. 2). In

the anterior region of the introvert, they are also cylindrical in form, but are much shorter than those on the introvert-basis, measuring about 0.04 mm in height (Text-fig. 3).

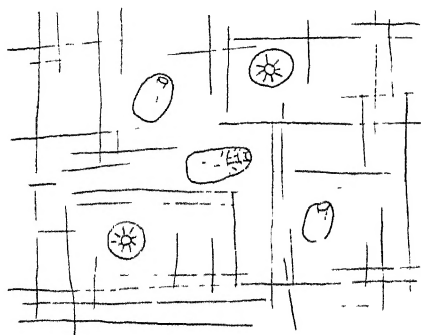
At the anterior end of the introvert, behind the tentacular crown, there are seen a number of hooks scattered irregularly. They are dark brown in colour and are bluntly pointed at the top. Each of these hooks (Text-fig. 4) is about



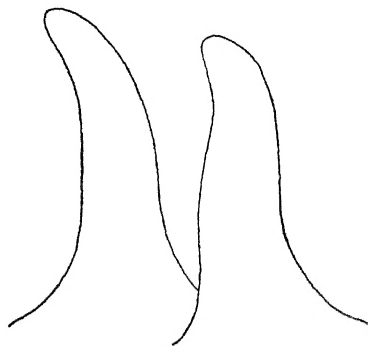
Text-fig. 1 *Phascolosoma vulgare* var. *tropicum* SLUITER. A papilla from the middle region of the introvert. $\times 580$.



Text-fig. 2. *Phascolosoma vulgare* var. *tropicum* SLUITER. Papillae found on the posterior end of the trunk. $\times 145$.



Text-fig. 3. *Phascolosoma vulgare* var. *tropicum* SLUITER. Papillae found on the introvert. $\times 145$.



Text-fig. 4. *Phascolosoma vulgare* var. *tropicum* SLUITER. Hooks found on the introvert. $\times 580$.

0.065 mm in height. It has a slightly curved apical tooth but is not provided with accessory tooth.

The tentacles are filamentous and numerous in number.

The longitudinal muscle layer of the body-wall is continuous. The inner surface of the body-wall shows a pearly lustre. Two pairs of slender retractor muscles are present. The ventral pair arises at the middle of the trunk, while the dorsal pair arises far anteriorly. Of these two pairs of muscles, the dorsal are narrower than the ventral. A single stout spindle-muscle arises from a point located behind the anus, and its posterior extremity is set free from the body-wall. There are three slender fixing-muscles. Two of them arise from the dorsal wall of the trunk, while the remaining one arises from the ventral wall of the same. All of these fixing-muscles are attached to the anterior portion of the intestinal convolution. Well-developed wing-muscles are found attached to the rectum near the anus. The intestinal convolution which coils around the spindle-muscle consists of about 40 spirals. It is free from the body-wall posteriorly. Polian tubules can not be detected on the Polian canal which passes along the dorsal side of the oesophagus. A pair of segmental organs are present. They are comparatively long tubes of grayish colour, and hung free into the body-cavity. The external apertures of these organs are situated almost at the same level with the anus. A pair of gonads is found lying along the basal part of each ventral retractor muscle. A rectal diverticulum is present upon the rectum.

Locality. Off Fudai, Pacific coast of North Japan.

Distribution. Sulu Island, Indian Archipelago (Siboga Expedition, Station 105).

Remarks. Three varieties of this species have been reported by SELENKA, SLUITER and LANCHESTER. They are *Phascolosoma vulgare* var. *astuta* SELENKA (1885, p. 11), *Phascolosoma vulgare* var. *selenkae* LANCHESTER (1905, p. 31) and *Phascolosoma vulgare* var. *tropicum* SLUITER (1902, p. 33).

Comparing the present specimen with the typical form and with these three varieties, I am aware that the present form very closely resembles the third variety *Phascolosoma vulgare* var. *tropicum* SELENKA with respect to the height of the hooks. And thus, I am inclined to identify this form with that variety.

4. *Phascolosoma margaritaceum* var. *antarcticum* MICHAELSEN.

(Pl. I, Fig. 3; Text-figs. 5-6)

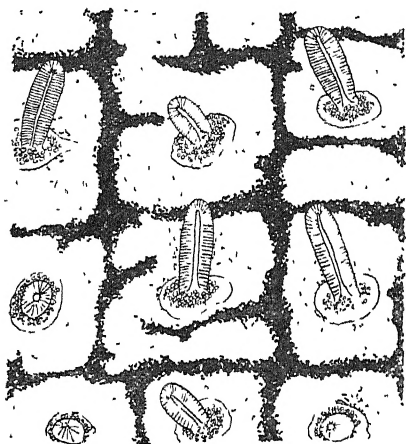
Phascolosoma margaritaceum var. *antarcticum* MICHAELSEN, FISCHER, 1928, p. 481, Pl. VI, Fig. 15.

Spec. No. N. 48; Station 560; N. Lat. 37° 26' 56", E. Long. 136°

22' 15"; Depth 172 m; Date July 22, 1930; Coll. KONISHI and WADA.

Two specimens were secured from a depth of 172 meters off the west coast of Noto Peninsula.

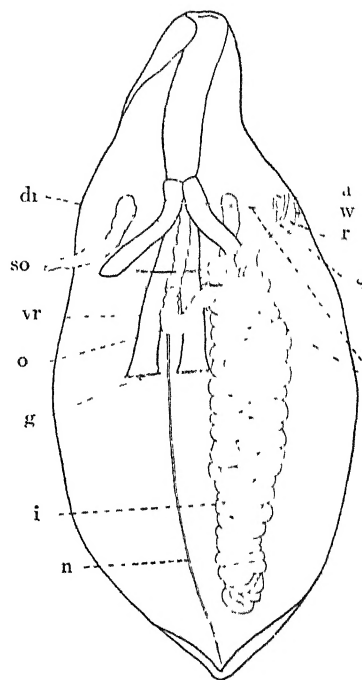
The body is of a somewhat elongated spindle-shape (Pl. I, Fig. 3). The trunk is about 40 mm long, while the introvert is slightly shorter



Text-fig. 5. *Phascolosoma margaritaceum* var. *antarcticum* MICHAELSEN. Papillae found on the posterior end of the trunk. $\times 145$.

than the trunk.

The skin is quite opaque being dirty pink colour when preserved in alcohol. It appears nearly smooth to the naked eye. But there are found numerous small papillae when observed under high magnification. The papillae found in the posterior region of the trunk, are cylindrical in form, measuring about 0.085 mm in height and 0.025 mm in diameter. In other regions of the body, they are all roundish being measured about 0.025 mm both in height and diameter. At the posterior region of the body, we see peculiar reticulation formed by pigmentation as shown in the Text-fig. 5. Hooks and spines are absent. Numerous finger-shaped tentacles exist encircling the mouth in several rows.



Text-fig. 6. *Phascolosoma margaritaceum* var. *antarcticum* MICHAELSEN. Specimen dissected. a, anus; dr, dorsal retractor muscle; f, fixing muscles; g, gonad; i, intestinal convolution; n, ventral nerve-cord; o, oesophagus; r, rectum; s, spindle-muscle; so, segmental organ; vr, ventral retractor muscle; w, wing-muscle. $\times \frac{3}{2}$.

The longitudinal muscle layer of the body-wall is continuous. Two pairs of the retractor muscle are present. The ventral pair (Text-fig. 6, vr) is attached to the body-wall at the level of the anterior one-third of the trunk, while the dorsal pair (Text-fig. 6, dr) is attached more anteriorly than the ventral. The fixing-muscles (Text-fig. 6, f) are three in number. One of the fixing-muscles springs from the anterior portion of the rectum and is attached to the inner surface of the body-wall near the ventral nerve-cord. The remaining two arise from the first whorl of the intestinal convolution and are attached to the body-wall near the left retractor muscle. A pair of broad wing-muscles (Text-fig. 6, w) are attached to the lateral wall of the rectum (Text-fig. 6, r) near the anus (Text-fig. 6, a). A simple Polian canal runs along the dorsal surface of the oesophagus (Text-fig. 6, o). The segmental organs (Text-fig. 6, so) consisting of two short tubes of a light grayish colour, are entirely free from the body-wall excepting the anterior extremities which are fastened to the body-wall. Their external apertures exist almost at the same level as the anus. A pair of sexual organs (Text-fig. 6, g) occur along the base of the ventral retractor muscles. Neither eye-spot nor rectal diverticulum is present.

Locality. Off Noto Peninsula, Japan Sea.

Distribution. Süd-Georgin; Cap Adare (Süd-Victoria-Land).

5. *Phascolosoma appendiculatum*, n. sp.

(Pl. I, Fig. 4, Text-figs 7-10)

Spec. No. S. 10; Station 342; N. Lat. $33^{\circ} 15' 20''$, E. Long. $133^{\circ} 48' 40''$; Depth, 288-527 m; Date, July 28, 1928; Coll. KAMIYA and MORIMOTO.

The collection contains three specimens of this new species. They are taken by dredge from the depth of 288-527 meters in Tosa Bay, Shikoku.

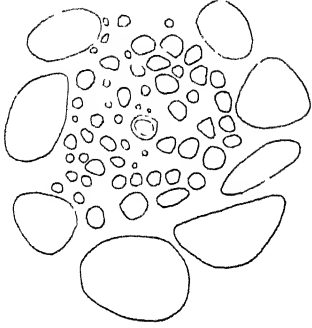
Of these three, one specimen which is selected as the type of this new species is well preserved, while the remaining two are imperfectly preserved.

In the type specimen (Pl. I, Fig. 4), the trunk is about 120 mm in length and is about 13 mm broad at the thickest part. The introvert which is much narrower than the trunk, measured about 70 mm in length.

At the posterior extremity of the body, there is found a peculiar tail-like appendage of about 10 mm long. It is a thin cord, having a uniform width of about 1 mm.

The skin of the trunk is thin, showing a dirty yellow colour. In the

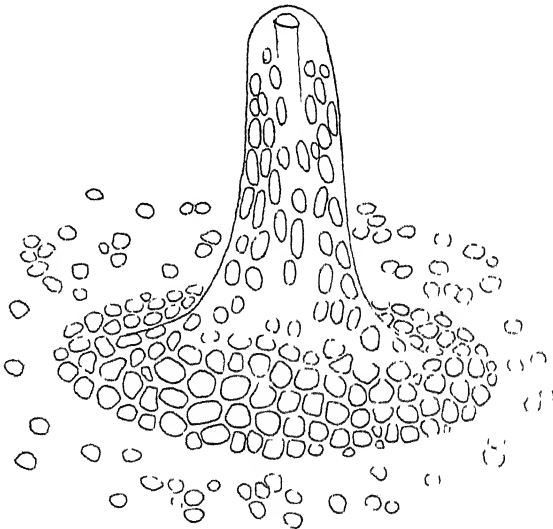
middle region of the body, the skin appears nearly smooth to the naked eye; while in both of the anterior and posterior region of the trunk, it appears rather rough and shows a dark brown colour. The whole surface



Text-fig. 7. *Phascolosoma appendiculatum*, n. sp. A papilla from the middle region of the trunk (Surface view) $\times 580$.



Text-fig. 8. *Phascolosoma appendiculatum*, n. sp. Papillae found on the introvert. $\times 115$.

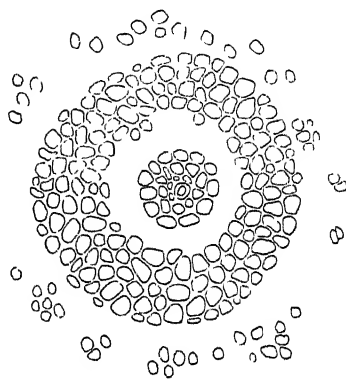


Text-fig. 9. *Phascolosoma appendiculatum*, n. sp. A papilla from the posterior end of the trunk. (Side view). $\times 580$

of the skin is covered by papillae well-developed. Those found in the middle region of the trunk are rather low, and are nearly circular in the surface view, with diameter of about 0.07 mm. Each of these papillae

consists internally of several large gland-cells and is covered externally by a great number of small chitinous plates. The papillae found on both the anterior and posterior regions of the trunk, are comparatively tall, measuring about 0.1 mm both in height and diameter. The papillae on the introvert are the tallest. They are conical in form and are more densely distributed than in other parts of the skin. Hooks or spines are absent. Tentacles which encircle the mouth are tolerably numerous.

The longitudinal muscle layer of the body-wall is continuous, and is not separated in bundles. The inner surface of the layer is smooth and lustrous in a grayish-white colour. Two pairs of the retractor muscles are present. Of these two the ventral pair is larger than the dorsal, and is attached at its posterior end to the body-wall, at the level of the anterior one-seventh of the trunk-length. The anterior part of these retractor muscles are connected with one another by means of mesenteries. The dorsal pair which is narrower than the ventral is attached anteriorly to the body-wall at a point situated in front of the roots of the ventral retractor muscles. A single stout spindle-muscle arises behind the anus and runs posteriorly with its extremity set free from the body-wall. Two fixing-muscles are present. Both of these spring from the body-wall near the root of the left dorsal retractor muscle, and are attached to the first whorl of the intestinal convolution. Wing-muscles are attached to the rectum near the anus. The intestinal convolution which coils around the spindle-muscle, consists of numerous spirals. It is free posteriorly from the body-wall. Polian canal is simple and does not bear the Polian tubules on it. Two long segmental organs of a dark reddish brown colour are present. They are entirely free from the body-wall except for the anterior part 2 mm long. The external apertures of these organs are situated almost at the same level with the anus. The rectal diverticulum can not be detected. The ventral nerve-cord is divided into two small branches at the posterior end of the body.



Text-fig 10 *Phascolosoma appendiculatum*, n sp. A papilla from the posterior end of the trunk (Surface view) $\times 435$.

Both of these spring from the body-wall near the root of the left dorsal retractor muscle, and are attached to the first whorl of the intestinal convolution. Wing-muscles are attached to the rectum near the anus. The intestinal convolution which coils around the spindle-muscle, consists of numerous spirals. It is free posteriorly from the body-wall. Polian canal is simple and does not bear the Polian tubules on it. Two long segmental organs of a dark reddish brown colour are present. They are entirely free from the body-wall except for the anterior part 2 mm long. The external apertures of these organs are situated almost at the same level with the anus. The rectal diverticulum can not be detected. The ventral nerve-cord is divided into two small branches at the posterior end of the body.

Locality. Tosa Bay, Japan.

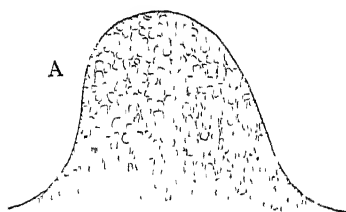
Remarks. This new species closely resembles *Phascolosoma flagriferum* described by SELENKA (1885, p. 13, Pl. III, fig. 17) and *Phascolosoma hudsonianum* CHAMBERLIN (1913, p. 41, Text-figs. 1-2), in presence of the characteristic caudal appendage. But it differs from the first in the number of the retractor muscles, and from the second in the shape of the papillae and in the situation of the attachment point of the retractor muscles to the body-wall. According to CHAMBERLIN (1913, p. 41, Text-figs. 1-2), the caudal appendage of *Phascolosoma hudsonianum* consists of a conical process of about 3.6 mm width measured at the base. In the present species, however, the caudal appendage, as already stated in description, is not conical but is like a cord having a uniform width of about 1 mm.

6. *Phascolosoma glossipapillosum*, n. sp.

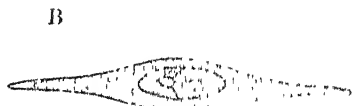
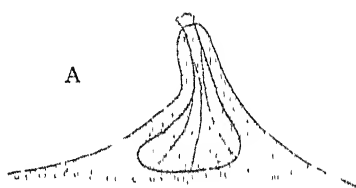
(Pl. I, Fig. 5; Text-figs. 11-14)

Spec. No. A. 618; Station 489; N. Lat. 35° 37' 10'', E. Long. 131° 02' 00''; Depth, 249 m; Date, Aug. 11, 1929; Coll. KONISHI and AIKAWA.

Several specimens were obtained from the depth of 249 meters off Hamada (Province of Iwami), Japan Sea.



Text-fig. 11. *Phascolosoma glossipapillosum*, n. sp. A papilla from the introvert. A, Side view; B, Surface view. $\times 116$.



Text-fig. 12. *Phascolosoma glossipapillosum*, n. sp. A papilla from the end of the trunk. A, Side view; B, Surface view. $\times 116$.

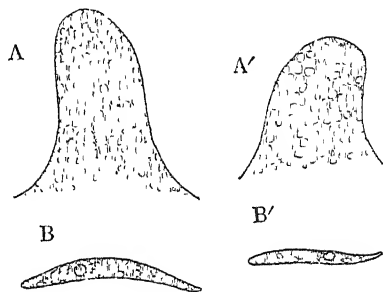
Of these specimens, a well-preserved one was selected as the type of this new species. The other specimens are rather imperfect being broken into fragments.

In the type specimen (Pl. I, Fig. 5), the trunk measures about 80 mm in length and about 12 mm in width at the widest part. It tapers posteriorly and terminates in a sharply pointed end. The introvert, which is much narrower than the trunk, has a uniform breadth. It measures about 60 mm in length and about 5 mm in width.

The colour of the skin is dark brown when preserved in alcohol. The posterior end of the trunk appears darker in colour than the other parts of body. The skin is thin, but entirely opaque. The circular muscles of the body-wall, especially those found in the posterior end of body, are very prominent showing externally numerous transversal ridges on the skin. The whole surface of the body is beset with numerous peculiar tongue-shaped papillae as shown in the Text-figs. 11-13. Each of these papillae is covered by numerous small chitinous plates and appears as an elongated ellipse in outline when viewed from above. Of all these papillae, those found on the introvert and on the introvert-basis are the largest, measuring about 0.24 mm in length, about 0.4 mm in major axis of the base and about 0.04 mm in the minor of the same. The papillae from the middle region of the trunk are smaller than those from the rest of the body. They measure 0.15-0.2 mm both in length and major axis of the base.

Numerous finger-shaped tentacles are present surrounding the mouth.

The longitudinal muscle layer of the body-wall is continuous. The inner surface of the body-wall is smooth and lustrous. Of the two pairs of the retractor muscles, the ventral pair is attached to the body-wall at the middle of the trunk (Text-fig. 14), while the dorsal pair is fixed to the same at the point located far anteriorly from the attachment-base of the ventral retractor muscles (Text-fig. 14, vr). A stout spindle-muscle (Text-fig. 14, s), which springs from the wall of the rectum behind the anus, is not fixed to posterior end of the trunk. A single fixing muscle (Text-fig. 14, f) which arises from the body-wall at a point situated on the left side of the ventral nerve-cord ends on the first whorl of the intestinal convolution (Text-fig. 14, i). The rectum (Text-fig. 14, r) is supported by the wing-muscle (Text-fig. 14, w) which is near the anus (Text-fig.



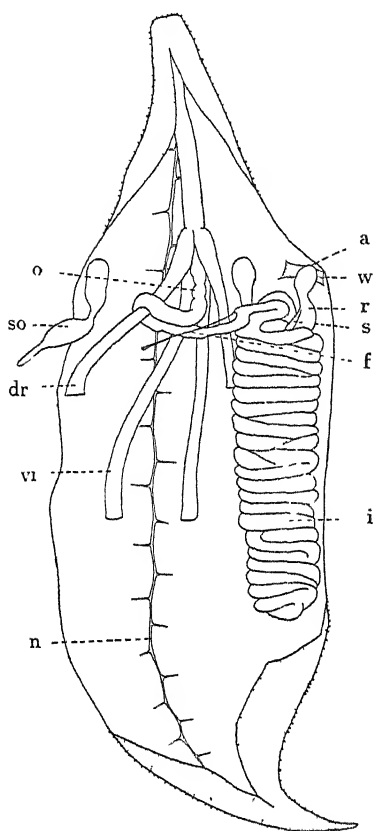
Text-fig 13. *Phascolosoma glossipapillosum*, n. sp. Papillae from the middle region of the trunk. A-A', Side view, B-B', Surface view. $\times 116$.

14, a). Intestinal convolution (Text-fig. 11, i) consists of about 25 spirals. There are found no Polian tubules on the Polian canal. A pair of

segmental organs (Text-fig. 11, so) are found running along both sides of the ventral nerve-cord (Text-fig. 14, n). They are small tubes of yellowish-brown colour and their external apertures are located almost at the same level with the anus. Each of these tubes is not fixed to the body-wall with its whole length but with the anterior end. The rectal diverticulum is absent. The ventral nerve-cord (Text-fig. 14, n) which is stretched upon the inner surface of the skin extending from the anterior extremity of the introvert to the posterior end of the trunk, is not closely attached to the body-wall by means of its side-branches, but is more or less separated from the latter.

Locality. Off Hamada (Province of Iwami), Japan Sea.

Remarks. Among the members of the genus *Phascolosoma* which are characterized by the possession of two pairs of retractor muscles and by the absence of hooks, we may not find such species as the present having papillae peculiarly shaped in tongue-like manner.



Text-fig. 14 *Phascolosoma glossipapillosum*, n. sp. Specimen dissected. a, anus; dr, dorsal retractor muscle; f, fixing-muscle; i, intestinal convolution; n, ventral nerve-cord; o, oesophagus; r, rectum; s, spindle-muscle; so, segmental organ, vi, ventral retractor muscle; w, wing-muscle. $\times 1$.

7. *Phascolosoma hyugense*, n. sp.

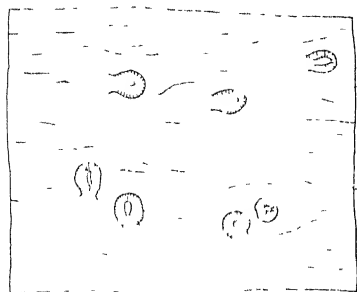
(Pl. I, Fig 6, Text-figs 15-16)

Spec. No. A. 436; Station 309; N. Lat. $31^{\circ} 41' 35''$, E. Long. $131^{\circ} 46' 40''$; Depth, 472 m; Date, July 14, 1928; Coll. KAMIYA and MORIMOTO.

Only one specimen (Pl. I, Fig. 6) was taken from the depth of 472 meters off Miyazaki in Kyû-hû, South Japan.

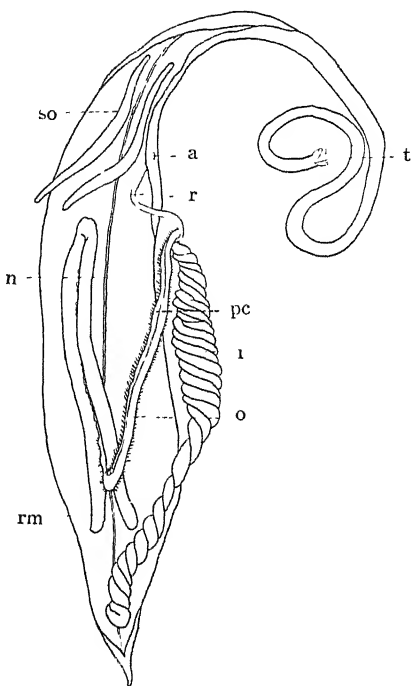
The trunk measures about 60 mm in length and 4 mm in thickness. The introvert which is slightly longer and much narrower than the trunk, is about 70 mm long and 1-2 mm thick.

The skin of the trunk is thin, yellowish grey in colour, and appears



Text-fig 15 *Phascolosoma hyugense*, n. sp. Papillae from the posterior region of the trunk. $\times 116$.

nearly smooth to the naked eye. The skin covering the introvert and the introvert-basis is rather thick. The papillae are numerous and are roundish, short, cylindrical in form with their ends rounded. They are about 0.04 mm in height and 0.03 mm in diameter at the base. They are nearly the same size throughout the whole surface of the body. The papillae are distributed most densely on the introvert-basis and at the posterior end of the trunk; while they are very sparsely scattered on the anterior region of the introvert. The features of each papilla are very similar to those of the papillae found in *Phascolosoma catharinae* F. MÜLLER (SELENKA, 1883, Taf. V, Fig. 62). Neither hooks nor spines are present. Very few tentacles (7-8) are found encircling the mouth in one row.



Text-fig. 16. *Phascolosoma hyugense*, n. sp. Specimen dissected. *a*, anus; *i*, intestinal convolution, *n*, ventral nerve-cord; *o*, oesophagus, *pc*, Polian canal with Polian tubules; *r*, rectum; *rm*, retractor muscle; *so*, segmental organ; *t*, tentacles. $\times \frac{3}{2}$.

The longitudinal muscle layer of the body-wall is continuous. The retractor muscles (Text-fig. 16, rm) consist of a single pair, and are attached to the body-wall at the level of the posterior one-fourth of the trunk-length. Their anterior parts come into contact with each other by means of mesentery. A stout spindle-muscle is present. Both of the fixing muscles and the rectal diverticulum can not be detected. The intestinal convolution (Text-fig. 16, i) which coils around the spindle-muscle, is set free from the body-wall posteriorly. The Polian canal (Text-fig. 16, pc) passes along the dorsal surface of the oesophagus (Text-fig. 16, o) giving off a great number of short blind tubules on its way. Two segmental organs (Text-fig. 16, so) of a grayish brown colour are present. They are about 20 mm in length and are entirely free from the body-wall except for the anterior extremity. The external apertures of the organs are situated about 10 mm distant anteriorly from the anus.

Locality. Off Miyazaki (Province of Hyûga), Kyûshû, Japan.

Remarks. This new species seems to be very closely allied to both *Phascolosoma catharinae* F. MÜLLER and *Phascolosoma martensi* COLLIN. But it differs from the first in the number and arrangement of the tentacles, and from the second in the length of the Polian tubules and in the position of the apertures of segmental organs.

8. *Phascolosoma noto*, n. sp.

(Pl. I, Fig. 7, Text-fig. 17)

Spec. No. N. 56; Station 559; N. Lat. 37° 20' 30'', E. Long. 136° 08' 45''; Depth 424 m; Date, July 22, 1930; Coll. KONISHI and WADA.

The collection contains two specimens of this new species. The first specimen (Pl. I, Fig. 7), which is selected as the type, is smaller but is better preserved than the second.

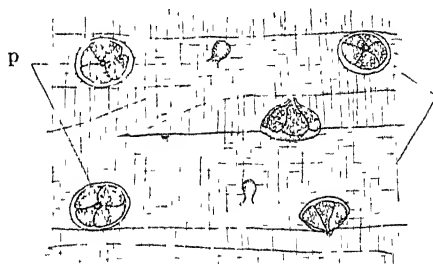
In the type specimen, the total body-length is about 55 mm. and the width is about 4 mm in the thickest part. The introvert is about one-third of the total body-length, and much narrower than the trunk.

The ground colour of the skin is dirty reddish brown, when preserved in alcohol. The body-wall is entirely opaque. The outer surface of the skin is beset with numerous papillae. The papillae on the introvert are pear-shaped and are distributed rather sparsely. They are very small in size measuring about 0.02 mm in height and about 0.03 mm in width. On the introvert-basis, we find a number of large flat papilla mixed with small pear-shaped papillae mentioned above. They are somewhat

elliptical in the surface view, measuring about 0.1 mm in major axis and 0.065 mm in the minor.

In the middle region of the trunk, the surface of the skin is provided with very small papillae scattered extremely sparsely. They resemble those on the introvert both in form and size. At the posterior end of the trunk, there are found large papillae mixed with the small pear-shaped papillae as in the case of the introvert-basis. Each of these large papillae is hemispherical in form, and measures about 0.05 mm in height and about 0.08 mm in diameter of the base. Hooks are absent on the introvert. Very few number of tentacles exist around the mouth.

To the naked eye, both the circular and longitudinal muscle layers of the body-wall seem to be continuous, but when the circular muscle layer is observed under high magnification we see that in the posterior region of the trunk it is divided into numerous narrow bands. The inner surface of the body-wall is smooth and lustrous. Two pairs of the retractor muscles are present. The ventral pair arises from the middle of the trunk close to the ventral nerve-cord, while the dorsal pair arises from a point located



Text-fig 17 *Phascolosoma noto*, n. sp. A piece of skin from the posterior region of the body c, circular muscle; p, papillae. $\times 116$.

at a short distance behind the anus. The intestinal convolution is traversed throughout by the spindle-muscle with its posterior end not fixed to the body-wall. There are two fixing muscles. They are composed of very fine muscle-strands and fix the anterior portion of the intestinal convolution to the body-wall. The rectum is supplied with the wing-muscles attached to its anterior portion. Polian canal is simple, and is not provided with Polian tubules. The segmental organs consist of two small sacs in pair. They are almost transparent and measure about 7 mm in length. Each sac of the organs is entirely free from the body-wall except its anterior extremity which is fixed to the latter. Their external openings are situated a short distance in front of the anus. Both the rectal diverticulum and the eye-spot are not detected.

Locality. Off Noto Peninsula, Japan Sea.

Remarks. This new species somewhat resembles *Phascolosoma solita*-

rum SLUITER and *Phascolosoma mausoni* BENHAM. But it differs from both of these species in the form of the papillae found on the body-wall.

9. *Phascolosoma signum*, n. sp.

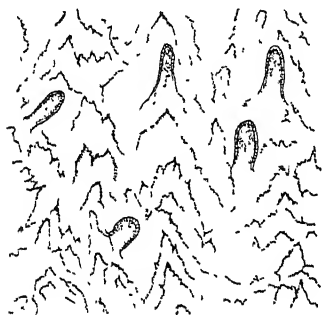
(Pl. I, Fig. 8; Text fig. 18)

Spec. No. N. 62; Station 550; N. Lat. $36^{\circ} 19' 35''$, E. Long. $135^{\circ} 34' 28''$; Depth, 658 m; July 20, 1930; Coll. KONISHI and WADA.

Three specimens of this new species were obtained from the depth of 658 meters in Wakasa Bay.

Of these three, one specimen which was preserved most perfectly is selected as the type of this new species.

In the type specimen (Pl. I, Fig. 8), the trunk measures about 45 mm in length and about 6 mm in thickness at the thickest part. The introvert, though much narrower than the trunk, is nearly as long as the trunk.



Text-fig. 18. *Phascolosoma signum*, n. sp. A piece of skin from the posterior end of the body. $\times 145$.

The skin is thin and shows a light gray colour when preserved in alcohol. The surface of the body-wall appears smooth to the naked eye, but there can be detected numerous small papillae when observed under high magnification. At the posterior end of the trunk, the skin is rugose and exhibits ripple-marks due to the pigment as shown in the text-fig. 18. The papillae found on the whole surface of the body excepting the posterior region, are spherical or pear-shaped, measuring about 0.025 mm in height and 0.02 mm in diameter. Those found on the posterior region of the trunk are tall

cylindrical in form measuring about 0.05 mm in height and 0.02 mm in diameter. Neither hooks nor spines are found on the introvert. Numerous finger-shaped tentacles are present. They are arranged in several radial rows around the mouth.

The longitudinal muscle layer is continuous and thus the inner surface of the body-wall is shiny. The retractor muscles occur in two pairs. The ventral pair originate at the level of the anterior one-third of the trunk-length close to the ventral nerve-cord. In the anterior parts, they are

fused together into a flat band. The dorsal pair are extremely slender, like the fixing-muscle mentioned below. They spring from the body-wall at the level situated slightly behind the anus. There are two fixing muscles which fasten the anterior portion of the intestinal convolution to the body-wall. Intestinal convolution consists of about thirty spirals, and is formed around the spindle-muscle with its posterior extremity not fixed to the body-wall. Along the dorsal side of the oesophagus runs the Polian canal. This canal is simple and is not beset with the Polian tubules. There exist two segmental organs, and their external apertures lie nearly at the same level as the anus.

Locality. Wakasa Bay, Japan Sea.

Remarks. This new species can be easily separated from the other members of the genus by the presence of the ripple-marks on the skin and by the extremely slender dorsal retractor muscles.

10. *Phascolosoma soyo*, n. sp.

(Pl I, Fig. 9, Text-figs. 19-21)

Spec. No. N. 59; Station 551; N. Lat. $36^{\circ} 22' 15''$, E. Long. $135^{\circ} 55' 00''$; Depth, 274 m; Date, July 20, 1930; Coll. KONISHI and WADA.

Spec. No. N. 54; Station 652; N. Lat. $41^{\circ} 27' 08''$, E. Long. $140^{\circ} 23' 00''$; Depth, 110 m; Date, Aug. 24, 1930; Coll. AIKAWA and FUJITA.

Spec. No. N. 52; Station 585; N. Lat. $36^{\circ} 59' 10''$, E. Long. $137^{\circ} 27' 15''$; Depth, 552 m; Date, Aug. 2, 1930; Coll. KONISHI and WADA.

This new species is represented in the collection by ten specimens which were secured from three different stations.

The dimensions of the body measured in these specimens are shown in the following tables.

TABLE I. (Specimens from Station 551).

	Trunk		Introvert	
	Length	Width	Length	Width
Specimen No. 1.	80 mm	12 mm	60 mm (Protruded)	5-7 mm
Specimen No. 2. (Type)	60 mm	6-7 mm	40 mm („)	1.5-2 mm
Specimen No. 3.	50 mm	6-7 mm	15 mm (Partly protruded)	1.5-2 mm
Specimen No. 4	35 mm	4 mm	15 mm („)	1 mm

TABLE II. (Specimens from Station 652).

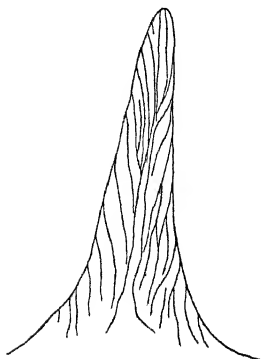
	Trunk		Introvert	
	Length	Width	Length	Width
Specimen No. 5.	45 mm	4 mm	30 mm (Protruded)	1.5-2 mm

TABLE III. (Specimens from Station 585).

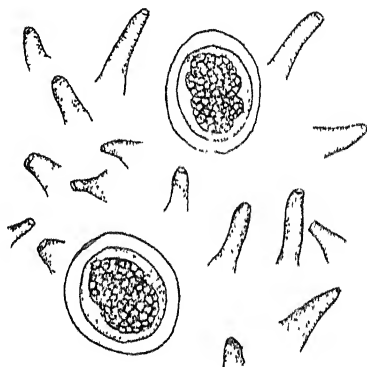
	Trunk		Introvert	
	Length	Width	Length	Width
Specimen No. 6.	150 mm	15 mm	30 mm (Partly protruded)	6-7 mm
Specimen No. 7.	110 mm	12 mm	32 mm (")	3-4 mm
Specimen No. 8.	125 mm	10 mm	28 mm (")	6-7 mm
Specimen No. 9.	90 mm	12 mm	46 mm (")	3-4 mm
Specimen No. 10.	90 mm	9 mm	30 mm (")	2-3 mm

As shown in the Table III, the specimens from Station 585 are on the whole larger in size than those from the other two stations.

The type specimen (Pl. I, Fig. 9), which is moderate in size and is



Text-fig. 19. *Phascolosoma soyo*, n. sp. A papilla from the anterior region of the trunk. (Side view). $\times 435$.



Text-fig. 20. *Phascolosoma soyo*, n. sp. Papillae found in the posterior region of the trunk. $\times 145$.

preserved much better than the others is reddish brown in the ground colour of the body-wall. The skin is rather thick and is entirely opaque.

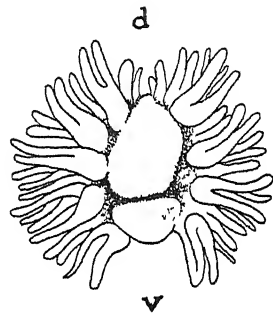
The outer surface of the body-wall appears nearly smooth to the naked eye, but there are found numerous papillae when observed under microscope (Text-figs. 19-20). The papillae found on the introvert are mostly conical, and some of them are sharply pointed at their tip. They measure about 0.05-0.13 mm in height and 0.03-0.1 mm in diameter at the base. In the middle region of the trunk, the papillae are flat and are very sparsely distributed. These papillae are elliptical in outline in surface view, and measures about 0.15 mm in major axis and 0.06 mm in minor axis. At the posterior region of the trunk, there exist two sorts of the papillae. The one is large and roundish, measuring about 0.07 mm in height and 0.1 mm in thickness; while the other is small and cylindrical, measuring about 0.07 mm in height and 0.02 mm in thickness (Text-fig. 20). Neither hooks nor spines are present on the introvert. Many finger-shaped tentacles exist encircling the mouth (Text-fig. 21).

Both of the longitudinal and the circular muscle-layers are continuous. The inner surface of the body-wall is thus smooth and has pearly lustre. Two pairs of the retractor muscles are present. The ventral pair arise at the level of the anterior one-third of the trunk-length, while the dorsal pair arise more anteriorly than the ventral. A single stout

spindle-muscle arises behind the anus, and its posterior extremity is set free from the body-wall. There are two slender fixing-muscles, each of which arises from the body-wall near the roots of the dorsal retractor muscles, and terminates on the first whorl of the intestinal convolution. The wing-muscles are found on both sides of the rectum attached at a point near the anus. The intestinal convolution consists of numerous spirals (more than 60 spirals). Polian canal is simple and bears no Polian tubules on it. The segmental organs are of short tubes, hanging free into the body-cavity. Their external apertures lie about 10 mm in front of the anus. A pair of gonads are found lying along the base of each ventral retractor muscle.

Localities. Off Mikuni (Fukui Prefecture), Japan Sea; Off Mikkaichi (Toyama Prefecture), Japan Sea; Tsugaru Strait.

Remarks. This new species is easily distinguished from the other



Text-fig. 21. *Phascolosoma soyo*, n. sp. Tentacular crown.
d, dorsal side; v, ventral side.
×9.

members of the genus *Phascolosoma* by the presence of sharply pointed conical papillae on the introvert, and also by the existence of two sorts of papillae in the posterior region of the trunk.

THEEL (1904, p. 78, Pl. 6, Figs. 77-79) reported *Phascolosoma abyssorum* which bears very tall conical papillae. But it has only two retractor muscles, while the present species has four of these. *Phascolosoma appendiculatum* first described in this paper is also provided with tall conical papillae distributed on the introvert as in the case of the present new species, but the former is differentiated from the latter by the presence of a tail-like appendage attached to the posterior extremity of the trunk.

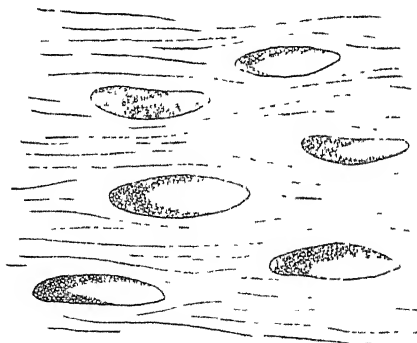
According to SLUITER (1912, p. 14, Pl. I, Fig. 4), it is described that *Phascolosoma iniquum* bears two sorts of papillae as in the case of the present new species. But they are distinguished from each other by the shape of the papillae they have.

11. *Dendrostoma ellipticum* n. sp.

(Pl I, Fig. 10, Text-figs. 22-25)

Spec. No. N. 94; Station 274; N. Lat. $34^{\circ} 44' 40''$, E. Long. $138^{\circ} 30' 40''$; Depth, 51 m; Date, July 2, 1928; Coll. KAMIYA and MORIMOTO.

Only one specimen (Pl. I, Fig. 10) of this new species was taken from a depth of 51 meters in Suruga Bay. The specimen was slightly injured



Text-fig. 22. *Dendrostoma ellipticum*, n. sp.
Papillae found in the posterior region of the trunk. $\times 116$.

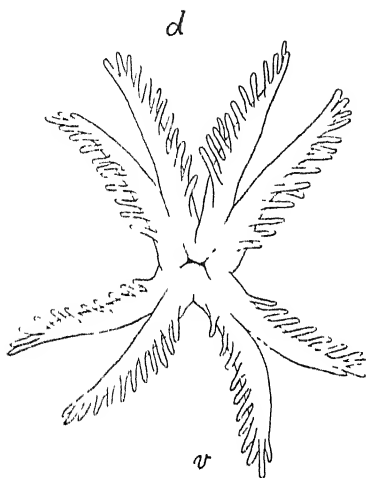


Text-fig. 23. *Dendrostoma ellipticum*, n. sp. A piece of skin from the anterior region of the introvert. $\times 461$.

the skin at the posterior region of the body, and a part of the intestine was forced out from the wound thus made.

The body has a total length of about 80 mm, and a thickness of about 5 mm. The introvert is about one-fifth of the total body-length.

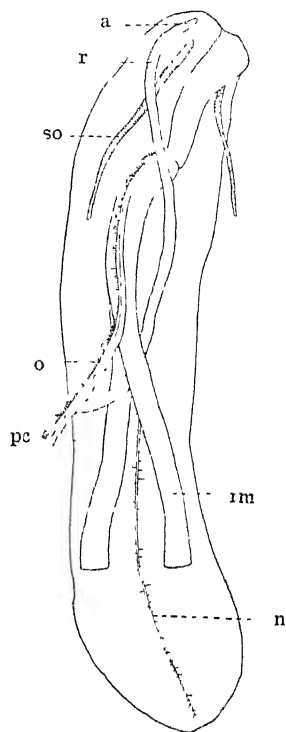
The skin is opaque, and is reddish brown in alcohol. It is covered with numerous papillae. They are more thickly distributed in the posterior region of the body than in the other regions of the same. These papillae are not roundish, but elongate elliptical in the surface view (Text-



Text-fig. 21 *Dendrostoma ellipticum*,
n. sp. Tentacular crown d, dorsal side,
v, ventral side.

fig. 22), measuring 0.1–0.17 mm in major axis and 0.03–0.05 mm in the minor. These papillae which are distributed at the posterior end of the body, are taller than those in the other parts of the body, and reach upto 0.03–0.06 mm in height. The papillae from the middle region of the trunk, are much inferior both in height and width to those from the other regions of the same. In the anterior region of the introvert, there are not found true papillae, but occur numerous small processes closely beset. Each of these processes is covered by numerous minute chitinous granules (Text-fig. 23).

The longitudinal muscles are continuous and thus the inner surface of the body-wall is shiny. The retractor muscles (Text-fig. 25, rm) occur in



Text-fig. 25. *Dendrostoma ellipticum* n. sp. Specimen dissected a, anus; n, ventral nerve-cord; o, oesophagus; pc, Polian canal with Polian tubules; r, rectum, rm, retractor muscle; so, segmental organ.
 $\times \frac{3}{2}$.

a single pair. They are long but slender, and are attached to the body-wall at the level of the posterior one-fifth of the trunk-length. The anterior portion of the retractor muscles is fused together into one piece at the anterior portion of the introvert. Numerous short Polian tubules are present upon the Polian canal (Text-fig. 25, pc) running along the dorsal side of the oesophagus (Text-fig. 25, o). The segmental organs (Text-fig. 25, so) which consist of two long tubes of a grayish colour, hang freely into the body-cavity, and their external apertures lie at a point slightly distant posteriorly from the anus.

Locality. Suruga Bay.

Remarks. Among the members of the genus *Dendrostoma*, there were hitherto known six species which are characterized by the possession of only one pair of the retractor muscles and by the absence of hooks on the introvert. They are *D. dehamata* KESTEVEN, *D. mythea* CHAMBERLIN, *D. perimeces* FISCHER, *D. peruvianum* COLLIN, *D. signifer* SELENKA et DE MAN, and *D. zostericola* CHAMBERLIN.

The present species is also furnished with the same characters as these species, however, it may be distinguished from these by the shape of the papillae as well as by the dimensions of the Polian tubules as shown in the following key.

Key to the species of *Dendrostoma*.

- I. Polian tubules extremely long.
 - D. perimeces* FISCHER.
 - D. peruvianum* COLLIN.
 - D. signifer* SELENKA et DE MAN.
- II. Polian tubules extremely short.
 - 1. Papillae are roundish in surface view.
 - D. dehamata* KESTEVEN.
 - D. mythea* CHAMBERLIN.
 - D. zostericola* CHAMBERLIN.
 - 2. Papillae are elongate elliptical in surface view.
 - D. ellipticum*, n. sp.

12. *Thalassema* sp. (?)

Spec. No. N. 43; Station 300; N. Lat. $31^{\circ} 18' 50''$, E. Long. $131^{\circ} 19' 30''$; Depth, 110 m; Date, July 11, 1928; Coll. KAMIYA and MORIMOTO.

The collection contains a small piece of proboscis which seems to belong to some Echiurid. It is band-like, being measured about 25 mm in length and about 5 mm in width, and is slightly convex on one side and concave on the other. The colour of this proboscis is grayish white when preserved in alcohol.

It is generally very difficult to determine the genus to which that animal belongs when there exist only its proboscis and missed its body. But judging from the features such as size, form, etc, it is highly probable that the present proboscis is owned by that animal belonging to the genus *Thalassema*.

13. *Priapulus bicaudatus* DANIELSSEN.

(Pl. I, Figs 11; Text-figs 26-31)

Priapulus bicaudatus, DANIELSSEN, 1868, p. 512⁴), THEEL, 1875, p. 23, 1906, pp. 18-19, Pl. I, figs. 3-6, Pl. II, figs. 9-10; HORST, 1881, pp. 13-38, Pls. II-III, SKORIKOW, 1901, FISCHER, 1914; 1921; 1922, p. 242; 1928, pp. 476-478.

Priapulopsis typica, KOREN and DANIELSSSEN, 1875.

Priapuloides typicus, KOREN and DANIELSSEN, 1881 & 1887.

Spec. No. A. 182; Station 134, N. Lat. 38° 17' 00'', E. Long. 141° 42' 00''; Depth, 139 m; Date, Nov. 21, 1925; Coll. KONISHI and YOSHIDA.

Spec. No. A. 186; Station 135, N. Lat. 38° 17' 00'', E. Long. 141° 45' 00''; Depth, 159 m; Date, Nov. 21, 1925; Coll. KONISHI and YOSHIDA.

Spec. No. A. 260; Station 130, N. Lat. 37° 07' 30'', E. Long. 141° 08' 40''; Depth, 104 m; Date, March 10, 1927; Coll. ASANO and MORIMOTO.

Spec. No. A. 326; Station 131, N. Lat. 36° 38' 15'', E. Long. 140° 53' 30''; Depth, 99 m; Date, March 12, 1927; Coll. ASANO and MORIMOTO.

Spec. No. A. 339; Station 129, N. Lat. 37° 21' 20'', E. Long. 141° 14' 00''; Depth, 82 m; Date, March 10, 1927; Coll. ASANO and MORIMOTO.

Spec. No. A. 537; Station 136, N. Lat. 38° 17' 00'', E. Long. 141° 56' 00''; Depth, 194 m; Date, Nov. 21, 1925; Coll. KONISHI and YOSHIDA.

Spec. No. S. 3; Station 45, N. Lat. 38° 52' 47'', E. Long. 142° 03' 30''; Depth, 349 m; Date, July 9, 1926; Coll. MARUKAWA and YOSHIDA.

Spec. No. S. 8; Station 141, N. Lat. 38° 17' 00'', E. Long. 142° 08' 00''; Depth, 680 m; Date, Nov. 24, 1925; Coll. KONISHI and YOSHIDA.

⁴ DANIELSSEN, 1868, Forh. ved. de Skandinav. Naturforskernes tiende Møde, Christiania, (I have been unable to get this DANIELSSEN's original paper).

Spec. No. S. 9; Station 335, N. Lat. $32^{\circ} 40' 50''$, E. Long. $133^{\circ} 11' 00''$; Depth, 399 m; Date, July 26, 1928; Coll. KAMIYA and MORIMOTO.

Spec. No. N. 4; Station 135, N. Lat. $38^{\circ} 17' 00''$, E. Long. $144^{\circ} 15' 00''$; Depth, 159 m; Date, Nov. 21, 1925; Coll. KONISHI and YOSHIDA.

Spec. No. N. 26; Station 23, N. Lat. $36^{\circ} 58' 00''$, E. Long. $144^{\circ} 21' 40''$; Depth, 170 m; Date, June 29, 1926; Coll. KAMIYA and NAKASHIMA.

Spec. No. N. 30; Station 115, N. Lat. $35^{\circ} 36' 30''$, E. Long. $140^{\circ} 46' 50''$; Depth, 18 m; Date, March 3, 1927; Coll. ASANO and MORIMOTO.

Numerous specimens of this interesting species were obtained from various localities as shown in the above list.

The largest specimen (Pl. I, Fig. 11), which was secured from a depth of 104 meters off Shiwoya-zaki measures about 75 mm in length and 11–15 mm in width; while in the smallest specimen, the body-length and the thickness measure about 15 mm and 5 mm respectively.

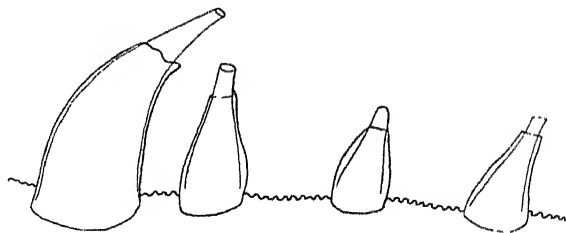
The introvert is nearly one-fourth of the total body-length, and is slightly thicker than the trunk.

At the posterior end of the trunk, and near the anus, there exist two respiratory organs (gills) consisting of numerous blind tubules (Text-fig. 31, ro).

On the ventral side of the trunk and close to the anus, a pair of minute pores are to be found, and these are the external apertures of the urogenital organs (Text-fig. 31, g).

The ground colour of the skin, when preserved in alcohol, is variable: in some specimens it appears dark reddish brown, while in the others it is grayish white, etc.

The body-wall is thick and opaque, and its outer surface is beset with



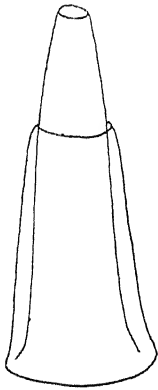
Text-fig. 26. *Priapulus bicaudatus* DANIELSEN.

Papillae found on the introvert. $\times 60$.

numerous papillae provided with a sharply pointed apex. The papillae on the introvert, are especially tall, and are arranged in 25 longitudinal rows.

Two of these rows placed on each side of the mid-ventral line are set close to each other, while the remaining rows are arranged at equidistance. On the outer surface of the trunk, the papillae are arranged in many transverse rings around the trunk. The papillae set in the posterior region of the trunk are taller than those found on the anterior region of the same.

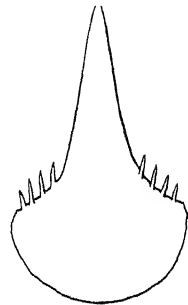
The inner surface of the pharynx, is furnished with numerous sharp teeth (Text-figs. 28-30). They are disposed in many sets of pentagons each of which being situated inside of the other. All the teeth set on one pentagon are of the equal size but differ from those found on the



Text-fig. 27. *Priapulus bicaudatus* DANIELSEN.
A papilla found on the posterior end of the body
× 116.



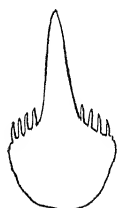
Text-fig. 28 *Priapulus bicaudatus* DANIELSEN.
A tooth (or hook) of the 2nd pentagon found on the wall of the pharynx
× 20.



Text-fig. 29. *Priapulus bicaudatus* DANIELSEN
A tooth (or hook) of the 3rd pentagon found on the wall of the pharynx. × 20.

other pentagons. On the first outermost pentagon ten teeth are found. Of these ten, every two are placed on each edge of the pentagon and not at each angle of the same. Each of these teeth is small and is somewhat triangular in shape provided with a small obtuse process not in the form of spine as in those from other pentagons. The second pentagon bears five teeth, each of which being placed at each angle. Each of these teeth is much forcible, and is consisted of one large central spine and three lateral ones arranged on each side of the central. The third pentagon, like the second, has five teeth. They are the largest comparing with those from other sets of pentagons, and each is provided with one large central spine and three or four small lateral teeth arranged on each

side of the central. The fourth pentagon has also five teeth. Each of these teeth is smaller than those on the second pentagon, and is provided with three or four lateral spines on each side of one large central spine. Thus advancing further, the teeth found on the more interiorly placed pentagons become smaller than those on the more exteriorly placed. And moreover, if roughly observed, they seem to be arranged quite irregularly without any definite order. In these smaller irregular teeth, inside the pentagons, the number of lateral spines increases up to ten, but decreases again towards the interior of the pharynx. The innermost teeth are represented by minute pointed warts. The height of the teeth on each pentagon is shown in the following table.



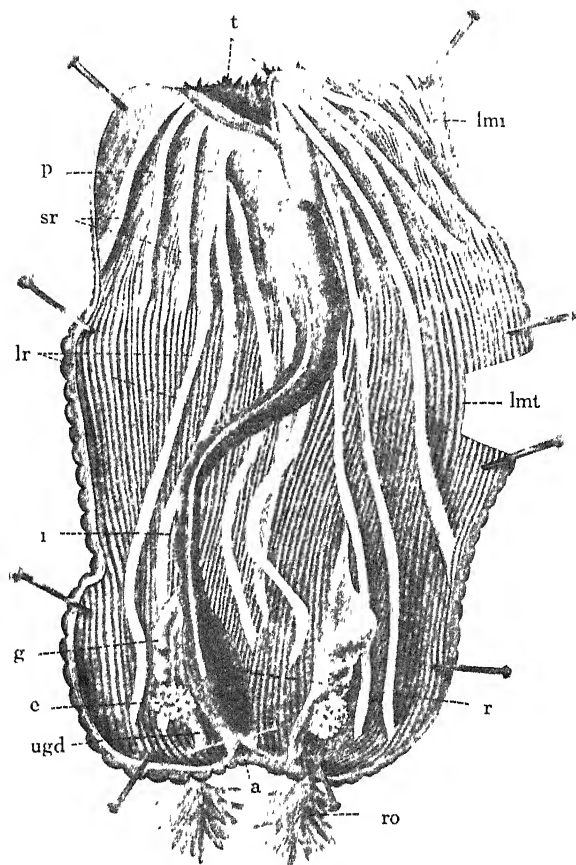
Text-fig. 30. *Priapulus bicaudatus* DANIELSEN
A tooth (or hook) of the 4th pentagon found on the wall of the pharynx $\times 20$.

TABLE IV.
Height of teeth on each pentagon.

	Spec. A	Spec. B	Spec. C.
First pentagon	0.3 mm	0.2 mm	0.1 mm
Second pentagon	1.5 mm	1.2 mm	0.8 mm
Third pentagon	2.0 mm	1.5 mm	1.0 mm
Fourth pentagon	1.3 mm	1.0 mm	0.8 mm
Fifth pentagon	1.0 mm	0.5 mm	0.5 mm

The tentacles are absent. The longitudinal muscles of the body-wall are divided into separate bundles. In the trunk region, the number of the muscle-bundles are 70-80; while in the region of the introvert, they are 25, corresponding with the number of rows of papillae on the outer surface of the introvert. Each of these bundles consists of short longitudinal muscle-bands running somewhat obliquely. The circular muscles are also divided into numerous separate bundles. There exist two sets of the retractor muscles, the one being much longer than the other. The longer retractor muscles are eight in number, and they all arise from the posterior region of the trunk and are attached to the anterior region of the introvert. Of these eight muscles, three arise from the ventral side of the body-wall, and one arises from the dorsal side of the same; while

the remaining four arise from the both lateral sides of the trunk (Text-fig. 31). The shorter retractor muscles are 10–11 in number. They arise from the anterior extremity of the introvert. The alimentary canal is represented by a short straight tube. Of the alimentary canal, it is distin-



Text-fig. 31. *Priapulus bicaudatus* DANIELSEN. Specimen dissected. *a*, anus, *e*, excretory organ; *g*, gonad; *i*, intestine; *lmi*, longitudinal muscle of the introvert; *lmt*, longitudinal muscle of the trunk; *lr*, longer retractor muscles; *p*, pharynx; *r*, rectum; *ro*, respiratory organ; *sr*, shorter retractor muscles, *t*, teeth (or hooks); *ugd*, urogenital duct. $\times \frac{3}{2}$.

guished the following three parts: 1) Pharynx, a short muscular sac, the inner surface of which is furnished with numerous sharp teeth. 2) Intestine, occupying the main part of the digestive canal. 3) Rectum, a short

but rather broad tube which is attached to the body-wall by means of a few number of fixing muscles. Situated on the ventral side of the posterior region of the trunk, there are found a pair of urogenital organs. Each of these organs is fastened to the body-wall by a mesentery throughout its whole length, and opens on the ventral side of the posterior extremity of the trunk.

Localities. All the specimens in the collection were obtained from the north Pacific near the Japanese coast (N. Lat. $38^{\circ} 52' 17''$ N. Lat. $32^{\circ} 40' 50''$).

Distribution. Finnmarken; Spitzbergen; Greenland; Norway; Ross Island; Wiide Bay; König-Karls-Land.

Remarks. This species was found for the first time at Varangerfjord (Ost-Finnmarken) and was described by DANIELSEN in 1869. From the sea of Japan, to my knowledge, on the occurrence of *Priapulid* it has not been reported.

According to THEEL (1906) and BALTZER (1931) we know that the southern limit of distribution of the present species is N. Lat. $62^{\circ} 14'$. But this time it was obtained at the locality situated along N. Lat. $32^{\circ} 40' 50''$.

LIST OF REFERENCES

- AUGENER, H. 1903. Beiträge zur Kenntnis der Gephyreen nach Untersuchungen der im Göttinger zoologischen Museum befindlichen Sipunculiden und Echiuriden Arch. Naturg., Jahrg. 69, Bd. 1, pp. 297-371, Pls. XIV XLVII.
- BAIRD, W. 1868. Monograph of the Species of Worms belonging to the Subclass Gephyrea. Proc. Zool. Soc. London, 1868, pp. 76-114, Pls. IX-XI.
- BALTZER, F. 1931. Priapulida. Handbuch der Zoologie, Bd. II.
- BENHAM, W. B. 1904. The Sipunculids of New Zealand. Trans. Proc. New Zealand Inst., Vol. 36, pp. 172-184, Pl. VII.
- . 1905. Further Notes on the Sipunculids of New Zealand. Trans. Proc. New Zealand Inst., Vol. 37, pp. 301-308, Pls. XV-XVI.
- CHAMBERLIN, R. V. 1920. (1). The Gephyrea collected by the Canadian Arctic Expedition, 1913-1918 Rep. Canad. Arctic Exped., Vol. 9, pp. 1-12, Text-figs. 1-4.
- . 1920. (2). Notes on Sipunculoidea of Laguna Beach. Jour. Entom. Zool. Claremont, Vol. 12, pp. 30-31.
- COLLIN, A. 1892. Gephyreen gesammelt von Herrn S. Dr. SANDER auf der Reise S. M. S. "Prinz Adalbert". Arch. Naturg., Bd. I, Heft 2, pp. 177-182, Pl. XI.
- . 1901. Die Gephyreen der deutschen Expedition S. M. S. "Gazelle". Arch. Naturg. Jahrg. 67, Beiheft (Martens), pp. 299-306.
- CUÉNOT, L. 1922. Sipunculien, Echiuriens, Priapulien. Faune de France, Vol. 4, pp. 1-29.
- . 1927. Contributions à la faune de bassin d'Arcachon, IX-Revue générale de la faune et bibliographie. Bulletin de la Station Biologique d'Arcachon, Tom. 24, pp. 299-305.

- DANIELSSSEN, D. C. og KOREN, J. 1880 Gephyreen fra den norske Nordhavsexpedition. *Nyt Mag. Natur vidensk.*, pp. 44-66, Pl. II
- . 1881. Gephyrea Den Norske Nordhavsexpedition 1876-1878, III Zoologie, pp. 1-58, Pls. I-VI.
- EHLERS, E. 1861 Ueber die Gattung *Priapulus* LAM. *Zeit. Wiss. Zool.*, Bd. 11, pp. 205-252.
- FISCHER, J. 1914 Die Sipunculiden der Nord und Ostsee unter Berücksichtigung von Formen des nordatlantischen Gebietes. *Wiss. Meeresuntersuch.*, Abt. Kiel, Neue Folge, Bd. 16, pp. 85-127, Pl. I, Text-figs. 1-9.
- FISCHER, W. 1892 Übersicht der von Herrn Dr. F. STUHLMANN auf Sansibar und an der gegenüberliegenden Festlandsküste gesammelten Gephyreen. *Jahrb. d. Hamb. Wiss. Anst.*, Bd. 9, pp. 80-89, Pl. I.
- . 1895. Die Gephyreen des Naturhistorischen Museums zu Hamburg. *Abhand. d. Geologie d. Naturwiss.*, Bd. 13, pp. 1-24, Pl. I.
- . 1896. Gephyreen. *Hamburger Margalhaensische Sammelreise*, pp. 1-7.
- . 1913. Über einige Sipunculiden des Naturhistorischen Museums zu Hamburg. *Mitt. nat. Mus. Hamb.*, Jahrg. 30, Beih. 2, pp. 93-101, Pl. I.
- . 1914. (1) Beiträge zur Kenntnis der Meeresfauna Westafrikas. herausgegeben von W. MICHAELSEN (Gephyrea) pp. 59-84, Pl. XI.
- . 1914. (2). Weitere Mitteilungen über die Gephyreen des Naturhistorischen Museums zu Hamburg. *Mitt. nat. Mus. Hamb.*, Jahrg. 31, Beih. 2, pp. 1-28, Pl. I.
- . 1917. Die Gephyreen ausbeute der Deutschen Tiefsee Expedition (1898-1899). *Zool. Anz.* Bd. 48, pp. 14-20.
- . 1919. Gephyreen der Südwestküste Australiens. *Zool. Anz.* Bd. 50, pp. 277-285, Text-figs. 1-6.
- . 1921. (1). Results of Dr. MJOBERG's Swedish Scientific Expeditions to Australia (1910-1913). XXVII. Gephyreen. *Svensk. Vet. Akad. Handl.* Bd. 61, No. 8, pp. 1-8, Text-figs. 1-6.
- . 1921. (2). Gephyreen der Antarktischen und Subantarktischen Meere. *Deutsche Südpolarexpedition. XVII, Zoologie (VIII)*, pp. 407-430, Text-figs. 1-4.
- . 1922. (1). Gephyreen des Arktischen Meere. *Wiss. Meeresuntersuch. Abt. Helgoland. N. F.* Bd. 13, pp. 229-246, Text-figs. 1-9.
- . 1922. (2). Gephyreen des Reichsmuseums zu Stockholm. *Arkiv für Zool. Stockholm*, Bd. 14, No. 19, pp. 1-39, Pls. I-IV.
- . 1926. (1). Sipunculiden und Echiuriden der Hamburger Südsee-Expedition, 1908-1909. *Mitt. aus dem Zool. Stat. u. Zool. Mus. in Hamburg.* Bd. 42, pp. 104-117, Pl. III.
- . 1926. (2). Sipunculoidea und Echiuroidea. Die Fauna Südwest-Australiens. *Ergebniss der Hamburger Südwest-australischen Forschungsreise 1905*, Bd. V, Lief. 3, pp. 199-214, Pl. II.
- . 1928. (1). Die Sipunculiden, Priapuliden und Echiuriden der Arktiks. *Fauna arctica Eine Zusammenstellung der arktischen Tierformen mit besondere Berücksichtigung des Spitzbergen-Gebietes auf Grund der Ergebniss der Deutschen Expedition in des Nördliche Eismeer im Jahre 1898*, Bd. V, Lief. 2, pp. 451-490, Pl. VI, Text-figs. 1-3.
- . 1928. (2). Über zwei neue *Siphonosoma*-Arten der Württ. Naturalien-Sammlung zu Stuttgart. *Zool. Anz.*, Bd. 76, Heft 316, pp. 137-143, Text-figs. 1-2.
- . 1928. (3). New Sipunculoidea from California. *Ann. Mag. Nat. Hist. (Zool.)*, Ser. 10, Vol. 1, No. 2, pp. 194-199, Pls. VI-VIII.
- GADD, G. 1911. Verzeichnis der Gephyreen des Kola-Golfes und zwei neue Species von

- Phascolosoma*. Trav. Soc. Nat. St-Petersburg C. R. T. 12, Livr. 1, (Abst.), pp. 102-105, Pl. I.
- GEROULD, J. H. 1913. The Sipunculids of the Eastern Coast of North America. Proc. U. S. National Museum, Vol. 14, pp. 373-437, Pls. LVIII-LXII, Text-figs. 1-16.
- HERUBEL, M. A. 1924. Quelques Echiurides et Sipunculides des côtes de Maroc et de Mauritanie. Bull. Soc. Sci. Nat. Maroc, Tom. 4, pp. 108-112, Text figs. 1-5.
- . 1925. (1). Quelques Echiurides et Sipunculides des côtes du Maroc. Bull. Soc. Sci. Nat. Maroc, Tom. 5, pp. 260-263.
- . 1925. (2). Description de *Phascolosoma reticulatum*, n. sp. Bull. Soc. Zool. France, Tom. 50, pp. 272-277, Text-figs. 1-6.
- HORST, R. 1881. Die Gephyrea gesammelt während der zwei ersten Fahrten des "Willem Barents". Nedel. Arch. Zool. Suppl., Vol. 1, pp. 1-42, Pls. I-III.
- HUTTON, W. K. 1903. On the Gephyrean *Phascolosoma teres*, n. sp. Proc. Zool. Soc. London, Vol. 1, pp. 29-41, Pls. VI-VIII.
- IKEDA, I. 1904. The Gephyrea of Japan. Jour. Col. Sci. Imp. Univ. Tokyo, Japan, Vol. 20, Art. 4, pp. 1-87, Pls. I-IV.
- . 1924. Further Notes on the Gephyrea of Japan with Descriptions of Some New Species from the Marshall, Caroline and Palau Islands. Jap. Jour. Zool., Vol. 1, No. 2, pp. 23-44, Pl. I.
- KEFERSTEIN, W. 1862. Beiträge zur Kenntnis der Gattung *Phascolosoma* in Untersuchungen über niedere Sectiere. Zeit. Wiss. Zool., Bd. 12, pp. 35-51, Pls. III-IV.
- . 1865. Beiträge zur anatomischen und systematischen Kenntnis der Sipunculoiden. Zeit. Wiss. Zool., Bd. 15, pp. 401-445, Pls. XXXI-XXXIII.
- . 1867. Untersuchungen über einige amerikanischen Sipunculiden. Zeit. Wiss. Zool., Bd. 17, pp. 44-54, Pl. VI.
- KESTIVEN, H. L. 1903. A New Species of *Dendrostroma*. Rec. Austral. Mus., Vol. 5, pp. 69-73, Pl. VII.
- LANCHESTER, W. F. 1905. (1). On a collection of Sipunculids made at Singapore and Malacca. Proc. Zool. Soc. London, Vol. 1, pp. 26-28.
- . 1925. (2). The Marine Fauna of Zanzibar and British East Africa, from Collections made by CYRIL CROSSLAND in the Years 1901-1902. Gephyrea. Proc. Zool. Soc. London, Vol. 1, pp. 28-35, Pl. I.
- . 1925. (3). On the Sipunculids and Echiurids collected during the "Skeat" Expedition to the Malay Peninsula. Proc. Zool. Soc. London, Vol. 1, pp. 35-41, Pl. II.
- MICHAELSEN, W. 1889. Die Gephyreen von Süd-Georgien nach der Ausbeute der Deutschen Station von 1882-1883. Jahrb. Hamb. Wiss. Anst., Bd. 6, p. 17, Pl. I.
- OSTROUMOV, A. A. 1909. Sur les géphyréens du nord de la mer Japon. Ann. Mus. Zool. Acad. Sci. St-Petersbourg. Tom. 14, pp. 319-324.
- PRASHAD, B. and AWATI, P. R. 1929. On a new species of the genus *Thalassema* from Bombay. Records of the Indian Museum, Vol. 31, Part 4, pp. 259-261.
- ROULE, L. 1898. Notice préliminaire sur les espèces des Gephyriens recueillies dans les Explorations sous-marines du Travailleur et du Talisman. Bull. Mus. d'Hist. Nat., pp. 384-387.
- SATÔ, H. 1930. Report of the Biological Survey of Mutsu Bay. 15. Sipunculoidea. Sci. Rep. Tôhoku Imp. Univ., Ser. IV, Vol. V, No. 1, pp. 1-40, Pls. I-IV, Text-figs. 1-15.
- . 1931. Report of the Biological Survey of Mutsu Bay. 20. Echiuroidea. Sci. Rep. Tôhoku Imp. Univ., Ser. IV, Vol. VI, No. 2, pp. 171-184, Text-figs. 1-4.

- SELENKA, E. 1883. On the Gephyreans of the Mergui Archipelago, collected for the Trustees of the Indian Museum, Calcutta. Jour. Linn. Soc. London, Vol. 21, pp. 220-222.
- SELENKA, E., DE MAN, J. G. und BÜLOW, C. 1883-1884. Die Sipunculiden. Reisen im Archipel der Philippine von Dr. C. SEMPER, Zweiter Teil, Wissenschaftl. Result., Bd. 1, Abt. 1, pp. 1-131, Pls. I-XIV.
- SELENKA, E. 1885. Report on the Gephyrea. Report on the Scientific Results of the Exploring Voyage of H. M. S. Challenger. Vol. 13, pp. 1-24, Pls. I-IV.
- SHIPLEY, A. 1898. Report on the Gephyrean Worms, collected by Mr. STANLEY GARDINER at Rotuma and Funafuti. Proc. Zool. Soc. London, Part 3, pp. 468-473, Pls. XXV-XXVII.
- 1899 (1). Notes on a collection of Gephyrean Worms found at Christmas Island by Mr. C. W. ANDREWS. Proc. Zool. Soc. London, Part 1, pp. 54-57.
- 1899. (2). A Report on the Sipunculoidea, collected in the Loyalty Island and New Britain. Willey's Zool. Results, Part 2, pp. 151-160, Pl. XVIII.
- 1899. (3). The List of the Gephyrean Worms of Funafuti. Australian Museum, Sydney, Memorie III, Part 8, p. 531.
- 1902. Sipunculoidea, with an Account of the New Genus *Lithacrosiphon*, Fauna and Geogr. Maldive Laccadive Archip., Vol. 1, pp. 131-140, Pl. VII.
- 1903. Report on the Gephyrea collected by Professor HERDMANN, at Ceylon in 1902. Rep. Gov. Ceylon Pearl Oyster Fish., 1903, pp. 169-176, Pl. I.
- SKORIKOW, A. 1902 (1). Gephyrea aus der zoologischen Ausbeute des Eisbrechers "Ermak" im Sommer 1901. Ann. Mus. Zool. Acad. Sci. St-Petersbourg, Tom 7, pp. 274-278.
- 1902 (2). Über die geographische Verbreitung einige Priapuliden. Zool. Anz. Bd. 25, No. 664, pp. 155-157.
- SLUITER, C. 1881. (1). Beiträge zu der Kenntnis der Gephyreen aus dem Malayischen Archipel. Nat. Tijds. v. Nederl. Ind., Bd. 41, Abt. 1, pp. 84-110, Pls. I-II.
- 1881. (2). Beiträge zu der Kenntnis der Gephyreen aus dem Malayischen Archipel. Nat. Tijds. v. Nederl. Ind., Bd. 41, Abt. 2, pp. 148-171, Pls. I-II.
- 1883. Beiträge zu der Kenntnis der Gephyreen aus dem Malayischen Archipel. Nat. Tijds. v. Nederl. Ind. Bd. 43, pp. 1-65, Pls. I-III.
- 1886. Beitrag zu der Kenntnis der Gephyreen aus dem Malayischen Archipel. Nat. Tijds. v. Nederl. Ind., Bd. 46, pp. 472-517, Pls. I-IV.
- 1890. Die Evertabraten aus der Sammlung der königlichen naturwissenschaftlichen Vereins in Niederländisch Indien in Batavia. Nat. Tijds. v. Nederl. Ind., Bd. 50, pp. 102-123, Pls. I-II.
- 1898. Gephyreen von Süd-Africa, nebst Bemerkungen über *Sipunculus indicus* PETERS. Zool. Jahrb. Abt. Syst., Bd. 11, pp. 422-450, Text-figs. A-B.
- 1900. Gephyriens provenant des Campagnes de l'Hirondelle et Princesse-ALICE (1886-1897). Resultant des Campag. Scient. accomp. sur son yacht par ALBERT Ier Prince Souverain de Monaco, Fasc. 15, pp. 1-29, Pls. I-III.
- 1902. Die Sipunculiden und Echiuriden. Siboga Expedition, Vol. 25, pp. 1-53, Pls. I-IV.
- 1912. Gephyriens provenant des Campagnes de la Princesse-Alice (1898-1910). Resultant des Campag. Scient. accomp. sur son yacht par ALBERT Ier Prince Souverain de Monaco, Fasc. 36, pp. 1-36, Pl. I.
- SOUTHERN, R. 1913. (1). Gephyrea of the Coast of Ireland. Fisheries Ireland Scient. Invest., No. 3, pp. 1-46, Pls. I-VII.
- 1913. (2). Clare Island Survey, Part 49, Gephyrea. Proc. Irish Acad., Vol. 31, No. 49,

p. 6, Pl I

- TEN BROEKE, A 1925. Westindischen Sipunculiden und Echiuriden. Resultaten einer Reis van Dr. C. J VAN DER HORST in 1920. Bijdrag. Direk. Atl. 24, pp. 1-16, Text-figs 1-25.
- THEEL, H 1875 Etude sur les Gephyriens inermes des mers de la Scandinavie, de Spitz berg et du Groenland. Bihang till K. Svenska Vet. Akad. Handling., Bd 3, No 6, pp 1-30, Pls. I-IV.
- 1905. Northern and Arctic Invertebrates in the Collection of the Swedish State Museum, I. Sipunculids. Bihang till K. Svenska Vet. Akad. Handling., Bd. 39, No. 1, pp 1-130, Pls. I-XV.
- 1911. Priapulids and Sipunculids dredged by the Swedish Antarctic Expedition 1901-1903, and the Phenomenon of Bipolarity. Bihang till K. Svenska Vet. Acad. Handling, Bd. 47, No. 1, pp. 1-36, Pls. I V.

EXPLANATION OF PLATE I.

- Fig. 1. *Sipunculus nudus* LINNAEUS. $\times 1$.
- Fig. 2. *Phascolosoma vulgare* var. *tropicum* SMITTER. $\times 1$.
- Fig. 3. *Phascolosoma margaritaceum* var. *antarcticum* MICHAELSEN. $\times 1$.
- Fig. 4. *Phascolosoma appendiculatum*, n. sp. $\times 1$.
- Fig. 5. *Phascolosoma glossipapillosum*, n. sp. $\times 1$.
- Fig. 6. *Phascolosoma hyugense*, n. sp. $\times 1$.
- Fig. 7. *Phascolosoma noto*, n. sp. $\times 1$.
- Fig. 8. *Phascolosoma signum*, n. sp. $\times 1$.
- Fig. 9. *Phascolosoma soyo*, n. sp. $\times 1$.
- Fig. 10. *Dendrostoma ellipticum*, n. sp. $\times 1$.
- Fig. 11. *Priapulius bicaudatus* DANIELSEN. $\times 1$.

Fig 1



Fig 1

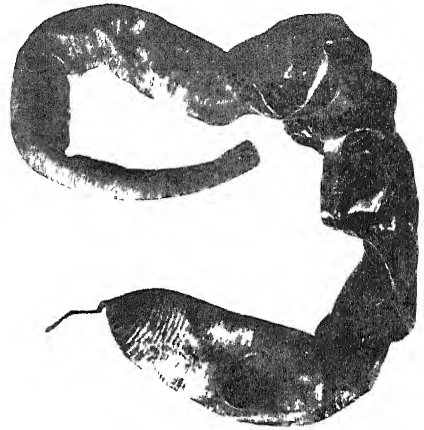


Fig 3



Fig 2



Fig 5



Fig 7



Fig 6.

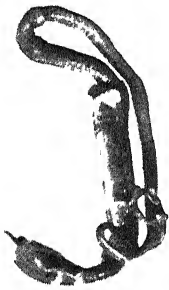


Fig. 8

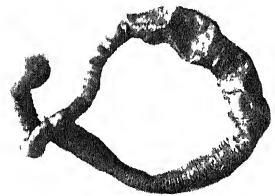


Fig. 11

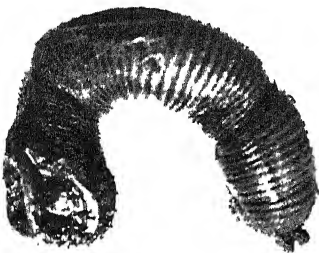


Fig. 10



Fig. 9.



ON THE MECHANISM OF FERTILIZATION AND DEVELOPMENT WITHOUT MEMBRANE FORMATION IN THE SEA URCHIN EGG, WITH NOTES ON A NEW METHOD OF ARTIFICIAL PARTHENOGENESIS*

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(With 10 figures)

(Received March 30, 1934)

I

Since the discovery of the methods of artificial parthenogenesis it became an important problem in biology to study the mechanism of initiation of development. One direction of investigation is to find out many new conditions which cause the artificial parthenogenesis and to establish an universal idea of changes, which take place in developing eggs. Another direction is to study the causal significance of a certain phenomenon which is occurred by altering the normal program in development by artificial means. In 1930 A. R. MOORE showed that the egg of the sea urchin can be fertilized and caused to develop without the formation of the fertilization membrane and the hyaline membrane, if the unfertilized egg first be treated with a solution of non-electrolyte, either urea or glycerine, and then fertilized. His observations suggest that the hyaline membrane is not the active cause of cell division and that the membrane is useful to the formation of blastula. Further, he showed that if ions of alkaline earth metals are added to the solution of non-electrolyte in sufficient amount, they protect the egg against the loss of capacity to form the membrane. From these facts the questions arise; whether the loss of the membrane forming capacity is the result of diffusion of something from the egg; or if not so, how can we describe the physiological condition of the egg which has lost the capacity to form the membrane?

II

It is well known, that butyric acid is a parthenogenetic activator on the unfertilized sea urchin egg. If the treatment with the butyric acid

* Contribution from the Marine Biological Station, Asamushi, Aomori-Ken. No. 112.

is sufficient, the egg forms the membranes, which are the essential feature in the activation of the egg by the spermatozoon. But if the effect of butyric acid is greater, the egg refuses to form the membranes, when returned to normal sea water. In autumn of 1933 at Asamusi I observed that the sea urchin eggs, which were previously treated with butyric acid, can be fertilized and that they develop without the formation of membranes. The results of my experiments are as follows.

In the first series of my experiment at Asamusi, I washed the unfertilized egg of *Strongylocentrotus nudus* (A. AGASSIZ) with sea water containing six per cent of N/10 aqueous solution of butyric acid for (1) five seconds, (2) ten, (3) twenty, (4) thirty, (5) forty, (6) sixty, (7) one hundred and twenty, and (8) three hundred seconds, respectively. They are then put into normal sea water. All or the majority of the eggs treated for twenty seconds with the acid form membranes. But in the longer or shorter treatment the eggs did not form the parthenogenetic membranes. Therefore the optimum effect of butyric acid as to the parthenogenetic activation for the egg of *Strongylocentrotus nudus* is twenty seconds at a room temperature of 20°C.

In the second series of experiment the eggs from the same female were treated with butyric acid as before, and they were then put into the sperm sea water. The egg treated for less than twenty seconds formed a normal fertilization membrane. By treatment for forty to sixty seconds the eggs were fertilized, but they did not show a well elevated fertilization membrane. And as a result, the blastomeres of these eggs were packed closely within a "firm" hyaline membrane, which is perhaps made of a poorly elevated fertilization membrane and a true hyaline membrane. After treatment for two minutes neither the fertilization membrane nor the hyaline membrane was formed by insemination. The egg began

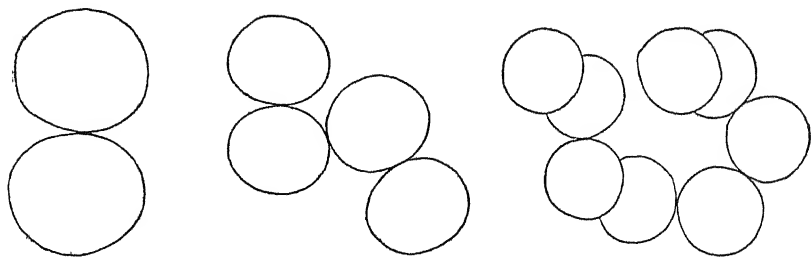


Fig. 1. Cleavage pattern of the egg of *Strongylocentrotus nudus*. The membrane formation is inhibited by treating with the butyric acid sea water for twenty minutes.

the first cleavage one and a half hours after fertilization with a division rate of a normal egg. The first two blastomeres are spherical (Fig. 1). They lie side by side on the bottom of the dish. In a few cases a feeble protoplasmic bridge was observed between them. In the second cleavage four blastomeres formed a chain. The two blastomeres at the free ends of the chain are connected to the inner pair of the blastomeres with simple protoplasmic bridges. In the following segmentation the blastomeres formed an irregular clump. After treatment for five minutes all eggs cytolysed.

The above facts describe a new similarity of the effect of butyric acid to that of the isotonic urea solution, which was studied by A. R. MOORE ('30 a, etc). If the egg is treated with the butyric acid sea water for a sufficient time, which is six times as long as that required to form the parthenogenetic membranes, it loses the capacity to form the membranes by a spermatozoon. I could find no difference between these figures and those of the urea treatment in MOORE's experiment.

III

The above experiment was repeated on the eggs of other species of sea urchins, *Pseudocentrotus depressus* (A. AGASSIZ) and *Strongylocentrotus pulcherrimus* (A. AGASSIZ), at Misaki. The results were the same as before.

The eggs of these species were put from one to thirty minutes into a mixture of 100 cc of sea water plus 6 cc N/10 butyric acid and then were put into normal sperm sea water of the species. The egg of *Pseudocentrotus depressus* when treated for seven to ten minutes with butyric acid at a room temperature of 13°C lost the capacity to form

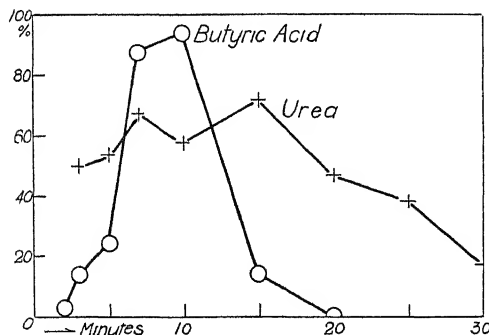


Fig 2. *Pseudocentrotus depressus*. The rate of cleavage without forming the membranes caused by the effect of butyric acid or the isotonic urea solution.

the membranes in 90 per cent. After a treatment for fifteen minutes the cell division was inhibited (Fig. 2). On the egg of the same female the effect of the urea solution was compared. The egg was put into 1 mol. urea solution, which is pH 8.3, for three to thirty minutes, and was fertilized in the normal sea water as before. After treatment for three to twenty minutes more than fifty per cent of the egg lost the capacity to form the membranes. And even the eggs treated for thirty minutes in seventeen per cent of the same, showed the cleavage without membrane formation (Fig. 2). Therefore, the effect of the urea solution is not severe in comparison with that of the butyric acid sea water.

When the eggs of *Strongylocentrotus pulcherrimus* were treated for ten minutes with the butyric acid sea water, they showed the cleavage without membrane formation in ninety two per cent. Eggs all died by treatment for twenty minutes. After a treatment for less than two minutes they did not lose the capacity to form the membranes (Fig. 3). It was

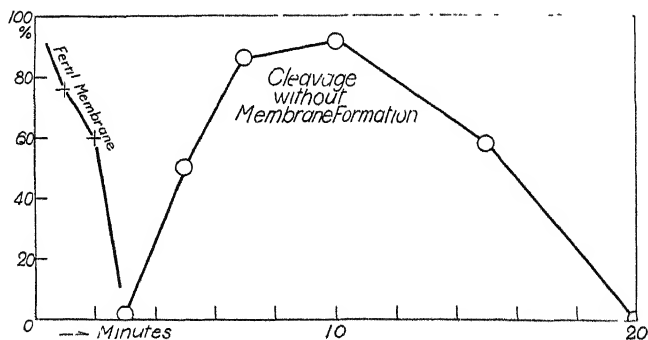


Fig. 3. *Strongylocentrotus pulcherrimus*. Rate of cleavage without forming the membranes caused by the effect of butyric acid

interesting that there is a slight difference between the effect of urea solution and that of butyric acid on the cleavage pattern of the egg. By the treatment with the urea solution a feeble protoplasmic bridge is formed between the blastomeres. And very often a chain of the blastomeres are observed (Fig. 4 a-c). But in the egg treated with butyric acid the protoplasmic bridge among the blastomeres looks firm. At the first cleavage the two blastomeres are spherical and lie apart from each other, as in the egg treated with urea. But the bridge between them is thick (Fig. 4 d-f). And in the second cleavage the new bridges are generally formed near by the first bridge. This results the branching of the first bridge (Fig. 4 g-h). In the following division some blastomeres

are also connected to the first bridge (Fig. 4 e). These figures are observed more frequently in the egg treated with butyric acid than in that of the urea treatment.

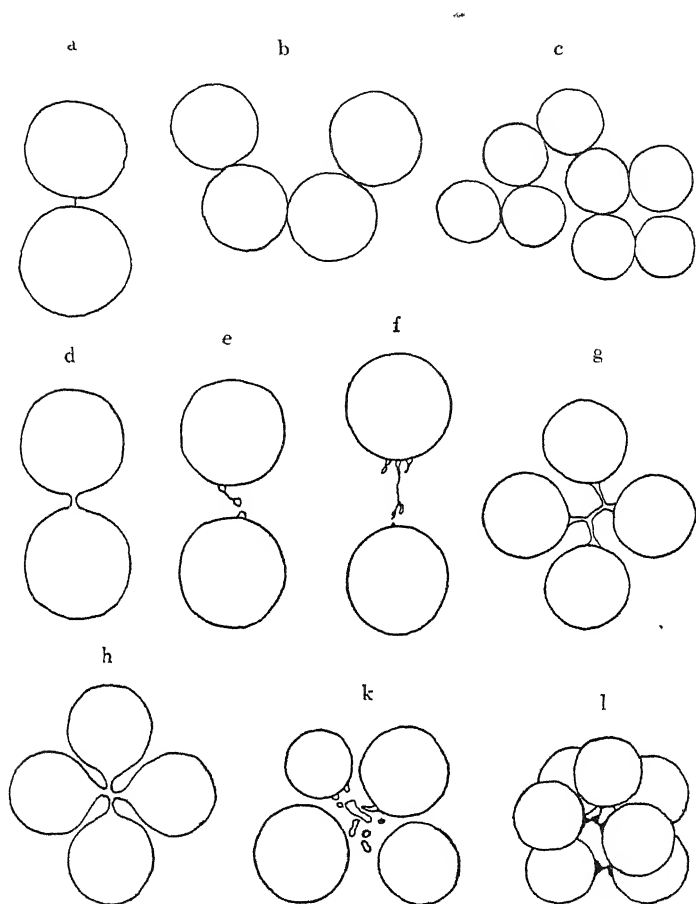


Fig. 4. Cleavage pattern of the eggs of *St. pulcherrimus*. The membrane formation is inhibited by the isotonic urea solution (a-c) or by butyric acid (d-i).

Needless to say, there is no essential difference of effects between these two reagents. A sufficient effect of the butyric acid causes the loss of the capacity to form the fertilization and hyaline membranes, just as the urea solution. But the only difference is, that in the egg treated with butyric acid the protoplasmic bridges look firm and thick in comparison with that of the urea treatment.

IV

As to the nature of the mechanism of inhibition of membrane formation the experiments described above show a great complexity. I have pointed out above that the egg lose the capacity to form the membranes by treatment with butyric acid as well as with the urea solution. These two solutions differ very much in their nature. Namely, the former is acidic and contains a great amount of metallic cations, because its main portion is sea water. On the contrary, the latter is alkaline, pH 8.3, and is poor in its amount of metallic ions. And yet, they equally cause the inhibition of the membrane formation. And it is at least possible to say that the egg will not be affected only by the absence of salts to lose the capacity of membrane formation. Because in my experiment of butyric acid the solution is isotonic and contains a balanced amount of salts.

The butyric acid sea water and the isotonic urea solution work on the already formed hyaline membrane in a different manner. I put the fertilized egg of *Pseudocentrotus depressus* into the butyric acid sea water or into the molecular solution of urea. In the butyric acid sea water the fertilization membrane shrinks a little. But it is not dissolved. The hyaline membrane remains unaffected by butyric acid even after an hour. The result in the urea solution is different. The fertilization membrane did not dissolve in the urea solution. But the hyaline membrane was dissolved within a few seconds in the urea solution. This fact suggests that the properties of the already formed hyaline membrane do not make known the nature of the state of the substance, which will become the hyaline membrane by being secreted outside the cytoplasm.

In 1932 A. R. MOORE showed that the effectiveness of the non-electrolyte solution in causing the loss of power to form the fertilization and hyaline membranes in the sea urchin eggs is related to the hydroxyl ion concentration of the solution, and that this destructive action of the hydroxyl ion with reference to the premembrane stuff is antagonized and may be completely inhibited by the cations of alkali and alkaline earth series when they are added in the form of chlorides to the solution of non-electrolyte. From his results we know the hydrogen-ion concentration will play an important action in the non-electrolyte solution. On the other hand, as pointed out by GRAY ('22) and HOBSON ('27) the formation of the fertilization membrane is not perfect in the acidic sea water. This seems ostensibly to be comparable to the results of my experiments. But when sea water is acidulated with hydrochloric acid, the formation

of fertilization membrane is not perfectly inhibited even at pH 2.3 (HOBSON '27). Therefore, I must conclude that the above effect of butyric acid is not caused by its hydrogen ion concentration, but it is a specific action of butyric acid. And as I have pointed out above, this specific action of butyric acid is equal to that of the isotonic solution of urea about the destructive action of the membrane forming capacity of the egg. We know already by the experiment of LOEB that the butyric acid sea water is an excellent reagent to cause the artificial parthenogenetic membrane formation. From these facts it is suggested that the isotonic solution of urea might also affect as a parthenogenetic activator, and that it might cause the parthenogenetic membrane formation in the sea urchin egg by treating for a suitable time with the urea solution.

V

To test the parthenogenetic activation effect of the isotonic urea solution the following experiment was carried out. The egg of *Strongylocentrotus nudus* was put into one molecular solution of urea for ten seconds and then into filtered sea water. All eggs formed the parthenogenetic fertilization membrane, hyaline membrane and monaster (Fig. 5). This experiment was repeated on the eggs of *Strongylocentrotus pulcherrimus* and *Pseudocentrotus depressus*. I could ascertain that the above fact is true also in these two species. The eggs of both species are activated to form the artificial membranes by treating them for seven to ten seconds with the molecular solution of urea (Figs. 6 & 7). The optimum time of exposure in the urea solution is much shorter than that of butyric acid. In the latter case the optimum is 80 seconds for the egg of *Pseudocentrotus* (Fig. 6) and forty to sixty seconds for *Strongylocentrotus pulcherrimus* (Fig. 7) respectively.

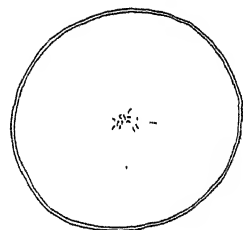


Fig 5 Parthenogenetic monaster in the egg of *St nudus* caused by the isotonic urea solution. Drawn from a fixed and stained material.

The activating effect of the isotonic urea solution agrees with that of butyric acid also in other points. As showed by LILLIE ('16 & '17) the swelling velocity of the egg in dilute sea water is much accelerated by the membrane formation, either in normal fertilization or in artificial activation. This is also true when the unfertilized egg is activated with the urea solution. The unfertilized egg of *Strongylocentrotus pulcherrimus*

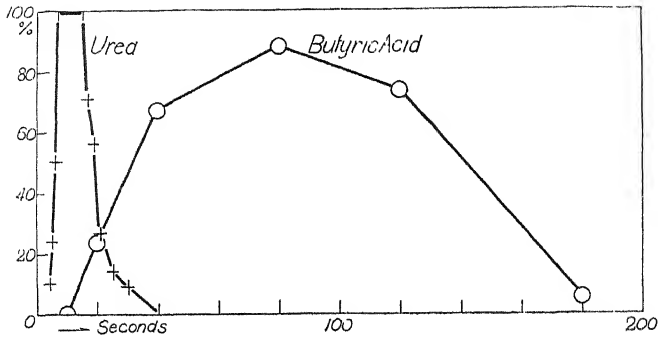


Fig. 6. *Pseudocentrotus depressus*. The rate of parthenogenetic membrane formation according to the time of washing in the isotonic urea solution or in the butyric acid sea water.

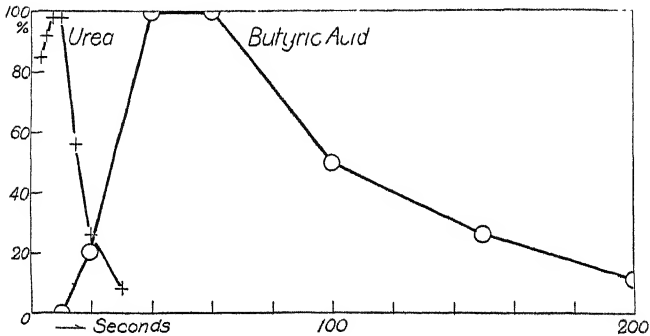


Fig. 7. *St. pulcherrimus*. Same as in Fig. 6.

was treated for seven seconds with the urea solution, or for sixty seconds with the butyric acid sea water, prepared by mixing 6 cc of N/10 butyric acid with 100 cc of sea water. In both cases the artificial membranes were formed in 100 per cent. After thirty minutes of the membrane formation the swelling velocity of the egg was tested in fifty per cent sea water. The result was a large swelling velocity in the eggs treated either with the urea solution or the butyric acid sea water in comparison with that of a control of unfertilized egg (Fig. 8). This fact shows the similarity of the effect of urea solution and butyric acid in their quality.

Moreover, the egg of *Strongylocentrotus pulcherrimus* develops to a gastrula when it is treated at first with the urea solution for seven seconds and then, ten minutes after this, treated for sixty minutes with a hypertonic sea water, which is made by mixing 5 cc of $2\frac{1}{2}$ M NaCl to 100 cc of sea water. The rate of larvae formed in this way are given in figure 9.

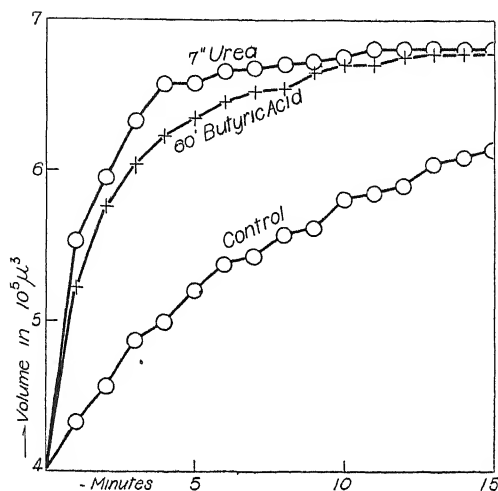


Fig. 8. *St. pulcherrimus*. Swelling velocity of the eggs in 50% sea water. The volume of the eggs was plotted against the time in the dilute sea water. The swelling velocity of the unfertilized eggs, which are previously washed either with the isotonic urea solution or with the butyric acid sea water, are compared with that of the unfertilized control egg. Each curve represents the mean value of two eggs respectively.

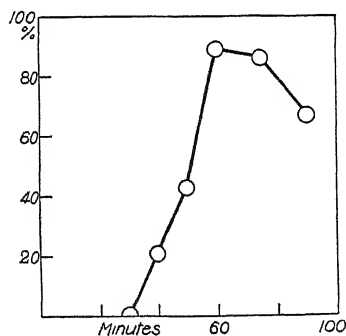


Fig. 9. *St. pulcherrimus*. The rate of parthenogenetic larvae formed by means of the urea method.

The optimum time of exposure to the hypertonic sea water is equal to the case, when the egg was activated at first with butyric acid. This shows that the isotonic urea solution is a parthenogenetic activator for the sea urchin egg, and that the effect of this solution is equal in its quality to butyric acid.

VI

In the foregoing chapters I have compared the effect of the molecular solution of urea with that of butyric acid. I conclude from the above facts that one molecular solution of urea is a parthenogenetic activator for the sea urchin egg. Now, we must examine the activating effect of the urea solution from the side of its osmotic pressure, because the osmotic pressure of a solution is one of the important factors on the parthenogenetic activation.

I made 2 mol., 1 mol. and 1/2 mol. aqueous solutions of urea. They are hypertonic, isotonic and hypotonic for the sea urchin egg respectively. In the isotonic solution the highest rate of membrane formation was obtained by treating the egg for seven to ten seconds. The rate of membrane formation decreases in relation to the length of time of washing in this solution; but even by the treatment for thirty seconds a few eggs formed the membranes (Fig. 10). In contrast with this, the tolerance of

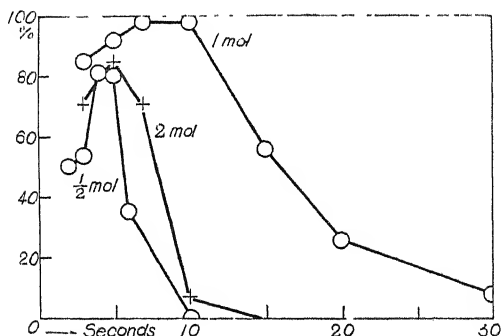


Fig. 10. *St. pulcherrimus* Effect of the osmotic pressure of the urea solutions on the rate of parthenogenetic membrane formation.

the egg for the membrane formation was very small, when it is treated with the hypertonic or hypotonic solution. In both cases the rate of membrane formation was highest at five seconds. When the treatment was longer or shorter than this, the rate decreased remarkably (Fig. 10).

This result shows that the isotonic solution is most suitable for artificial membrane formation among the above three solutions. In other words, the effect of hypertonic or hypotonic solution is severe in comparison with that of the isotonic solution. And, moreover, it suggests that the parthenogenetic activating effect of the urea solution is not simply caused by its osmotic pressure, because the effect of the hypertonic and hypotonic solutions are nearly similar.

VII

Discussion.—I described a new fact that the eggs of the sea urchins, *Strongylocentrotus nudus*, *St. pulcherrimus* and *Pseudocentrotus depressus*, lose the capacity to form the membranes, if they are washed with the butyric acid sea water. In comparing this with the results obtained by MOORE, I understand that the hydrogen ion concentration of the solution is not the first importance to cause the loss of the capacity of membrane formation of the egg. This view is supported also by the facts, that the egg does not lose perfectly the capacity of membrane formation by the acidic sea water, when hydrochloric acid is used (GRAY '22, HOBSON '27). Also the concentration of salts will have only an accessory meaning for this phenomenon. The presence of cations is not effective to protect the egg from the loss of the membrane forming capacity against the effect of butyric acid. In short, the development without membrane formation is not simply attributed to the concentration of hydrogen-ion or to the absence of metallic ions. Therefore, I conclude that the development without membrane formation is caused by the abnormal activation* of the egg.

The egg is activated with the isotonic urea solution as well as with butyric acid. The former causes the membrane formation, aster formation, increase of permeability and also the parthenogenetic development. These effects are analogous to that of butyric acid. Moreover, the loss of capacity of membrane formation can be caused by treating the egg for a long time either with the isotonic urea solution or with the butyric acid sea water. These phenomena are in the series of responses relating to the strength of effects of the reagents. The phenomena of the least effect are the parthenogenetic membrane formation. Both the urea solution and butyric acid do this by a treatment for a short time. Next, if the action of these solutions is longer continued, the egg will not elevate the fertilization membrane perfectly, although easily be seminated. In the case of the strongest effect, the capacity of membrane formation is lost, and thus the connections among the blastomeres become loose. And if we care for the fact that these two reagents are the parthenogenetic activators, it will be rational to consider the above series of responses of the egg as in accordance with the magnitude of stimulus to development. When the egg is previously activated sufficiently either with the urea solution or butyric acid and then inseminated, it suffers a greater stimulus of develop-

*The idea of activation of the egg is defined as a phenomenon of changes in a direction to development.

ment than the normal egg, because in this case the stimulus of a sperm is added to that of the foregoing parthenogenetic activator. Therefore, the loss of the capacity of membrane formation will be caused by the strongest stimulus, which is an additive effect of a sperm and of the parthenogenetic activation. If we remember the facts, that these two reagents are not unlike in their physico-chemical properties, we must be satisfied with the explanation, that the development without membrane formation will be caused by the additive effect of the stimuli of a sperm and of a parthenogenetic activator of a sufficient strength.

As pointed out by LILLIE ('10, '11), the sea urchin egg is activated when it is treated with an isotonic solution of pure salt. And the addition of small quantities of calcium chloride to the isotonic solution of sodium salts prevents the membrane formation and initiation of cell division which are typically induced by the pure solution. It will be expected that if the salts of sea water are added to the isotonic urea solution, the activating power of the urea solution will be canceled. But I did not carry out this experiment.

SUMMARY

1) The capacity to form the fertilization- and the hyaline-membrane is inhibited by previously activating the eggs of the sea urchins, *Strongylocentrotus nudus*, *Strongylocentrotus pulcherrimus* and *Psedocentrotus depressus* with the butyric acid sea water. In other words, the development without membrane formation is caused by adding the effect of a sperm to that of the parthenogenetic activators, such as the butyric acid sea water or the urea solution.

2) Isotonic, hypertonic and hypotonic urea solutions act as parthenogenetic activators on the sea urchin egg. The egg of *Strongylocentrotus pulcherrimus* develops to a gastrula when it is treated at first with the isotonic urea solution for seven seconds and then, ten minutes after this, treated for sixty minutes with the hypertonic sea water.

3) Both the butyric acid sea water and the urea solution are alike in their activating effect.

4) The butyric acid sea water and the urea solution work on the already formed hyaline membrane in a different manner. It is dissolved in the urea solution, but is not dissolved in the butyric acid sea water.

LITERATURE CITED

- GRAY, J. 1922 A critical study of the facts of artificial fertilization and normal fertilization. Q. J. micr Sci, LXVI, 419.
- HOBSON, A. D. 1927. A study of the fertilization membrane in the echinoderms Proc. Roy Soc Edinburgh, XLVII, 94.
- LILLIE, R. S. 1910 The physiology of cell division II. The action of isotonic solution of neutral salts on unfertilized eggs of *Asterias* and *Arbacia*. Amer J Physiol, XXVI, 106.
- 1911. The physiology of cell division III The action of calcium salts in preventing the initiation of cell division in unfertilized eggs through isotonic solutions of sodium salts Amer. J. Physiol., XXVII, 289.
- 1916 Increase of permeability to water following normal and artificial activation in sea urchin eggs Amer. J. Physiol, XL, 249.
- 1917. The conditions determining the rate of entrance of water into fertilized and unfertilized *Arbacia* eggs, and the general relation of changes of permeability to activation. Amer. J. Physiol, XLIII, 43.
- MOORE, A. R. 1930 . Fertilization and development without membrane formation in the eggs of sea urchin, *Strongylocentrotus purpuratus* Protoplasma, IX, 9.
- 1930 b Fertilization and development without fertilization membrane in the egg of *Dendraster eccentricus* Protoplasma, IX, 18.
- 1932 a The role of unantagonized cations in protecting the membrane forming function in the egg of the sea urchin Protoplasma, XV, 268.
- 1932 b The dependence of cytoplasmic structures in the egg of the sea urchin on the ionic balance of the environment. J cell. and comp. Physiol., II, 41.
- 1933 The relative values of cations in protecting the membrane forming capacity of the eggs of the echinoids, *Clypeaster japonicus* and *Temnopleurus hardwickii*. Sci Rep. Tôhoku Imp Univ 4th Ser. (Biol), VIII, 249
- MOORE, A. R. and M. M. MOORE 1931. Fertilization and development without membrane formation in the egg of the sea urchin (*Paracentrotus lividus*) Arch. de Biol, XLII, 375.
- MOORE, M. M. 1932 On the coherence of the blastomeres of sea urchin eggs. Roux' Arch., CXXV, 187.

ON THE COELOMIC CORPUSCLES IN THE BODY FLUID OF SOME INVERTEBRATES¹⁾

I. REACTION OF THE LEUCOCYTES OF A HOLOTHURID, *CAUDINA CHILENSIS* (J. MÜLLER), TO VITAL DYES²⁾

By

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(With 5 text-figures)

(Received March 31, 1934)

During the summer of 1932 I made some observations on the reaction of the leucocytes of a holothurid, *Caudina chilensis* (J. MÜLLER), to vital dyes. The work was carried on at the Asamushi Marine Biological Station of the Tōhoku Imperial University. I wish to express my hearty thanks to Prof. S. HATAI for his kind advice and valuable criticism on the present work.

MATERIAL AND METHODS

The animals were collected from the vicinity of the station, and kept in the aquariums which were filled with grains of fine sand.

As the vital dyes, I used trypan blue and carmine. These dyes were harmless enough to enable an amount to be administered which would produce visible staining in the corpuscles.

Trypan blue was used in the form of 0.1 per cent solution in sea water. The solution was boiled and filtered when cooled. Intraperitoneally the dosage for *Caudina* was 5 c.c. of 0.1 per cent solution per 100 g. body weight. Intense vital staining was obtained by three to four injections every other day.

Following Dr. MURATA's (Late Prof. of Osaka Imperial University) suggestion I used sodium carmine instead of lithium carmine. Sodium carmine is prepared by adding 4 per cent in weight of pure carmine to a saturated aqueous solution of sodium bicarbonate. The carmine dissolved readily, forming a purplish red solution. The solution should be boiled and filtered when cooled. This original solution was diluted just before

¹⁾The expenses of these investigations were defrayed by a grant from the Department of Education for which I express my indebtedness.

²⁾Contribution from the Marine Biological Station, Asamushi, Aomori-ken. No. 113.

administration about ten times with distilled water. *Caudina* received 2 c.c. of this solution per 100 g. body weight, and three to four injections at an interval of two days were always given. I also administered trypan blue and carmine in combination. In such cases the dosage of two solutions was just half of that above mentioned. After three days of the latest injection, the perivisceral fluid was drawn and examined under the oil-immersion lens.

OBSERVATIONS

Of the histological study on the coelomic corpuscles of *Caudina*, there is an excellent report by KAWAMOTO. He distinguished six kinds of corpuscles: red, white, minute, brown, fusiform, and crystal corpuscle. In these cells, in the present investigation, the red and crystal corpuscles showed negative reaction to the vital staining. In the supravital staining, however, the bright, brown granules of red corpuscles were stained by neutral red.

KAWAMOTO described two subtypes of white corpuscles. One contains many colorless spherules, and has lobed-shaped pseudopodia. Another kind of corpuscles move more actively and the spherules are more sparsely distributed than in the former type. KINDRED ('21) distinguished also two kinds of the white corpuscles of *Arbacia*. Leucocytes, the first type, are the most generalized cells in the perivisceral fluid and they are highly phagocytic, thrombogenic and scleroblastic. The amoebocytes with spherules, the second type, carry on none of these functions. He states in another paper ('26) that the leucocytes and amoebocytes have genetically equal origin, and the amoebocytes with spherules is inferred to arise from the leucocyte which has ingested food. It seems to me that the descriptions of the white corpuscles of *Caudina* and *Arbacia* are well concordant with each other: viz. the white corpuscles which belong to the first type of KAWAMOTO agree with KINDRED's amoebocyte with spherules, and KAWAMOTO's second type of white corpuscles with KINDRED's leucocytes. So I wish to apply the terms "leucocyte" and "amoebocyte with spherules" to the white corpuscles of *Caudina*.

KINDRED reported that no success was met with in the attempt to stain the leucocytes of *Arbacia* by the injection of vital dyes. In the present investigation of *Caudina*, however, fine results were obtained constantly with trypan blue and carmine. It is frequently seen that the cytoplasm of leucocyte was filled densely with dye granules (carmine or trypan blue, or both of them when they were introduced in combination).

The amoebocytes with spherules contain, of course, the dye granules not at all or very sparsely. Between these two extremes there exist various transitional stages of staining. The presence of these grades seems to be very reasonable, if the amoebocytes arise from the leucocytes as a result of active phagocytosis. For the leucocytes which have less ingested food will be able to devour more dye granules.

In the case of the injection in which trypan blue and carmine were introduced in combination, we can find the leucocytes in various stages of staining. Some are stained only with either trypan blue or carmine; others accumulated the granules of both dyes in various proportions. There were also corpuscles which contained the purple granules upon rare occasions.

A drop of perivisceral fluid of *Caudina* freshly drawn, when placed on a cover slip, immediately shows signs of agglutination. When the

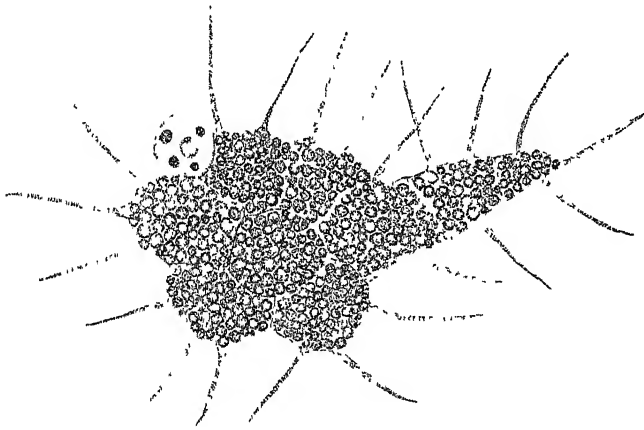


Fig. 1. The clotting formation of leucocytes. All cells are filled with the vital-dye granules. $\times 1100$.

animal was previously administered with vital dyes, the greater part of cells forming clotting are intensively stained with dyes (Fig. 1). This fact proves that the cells which partake the clotting formation are chiefly the leucocytes.

THEEL ('20-'21) reported the syncytium formation by the fusion of many "plasma-amoebocytes" of a holothurid, *Labidoplax buskii*. I also found occasionally the presence of fused leucocytes with pseudopodia (Fig. 2). The number of fused cells was, however, limited constantly to only two in the present case. So I suppose this fusion may be a result of amitotic division of leucocytes.

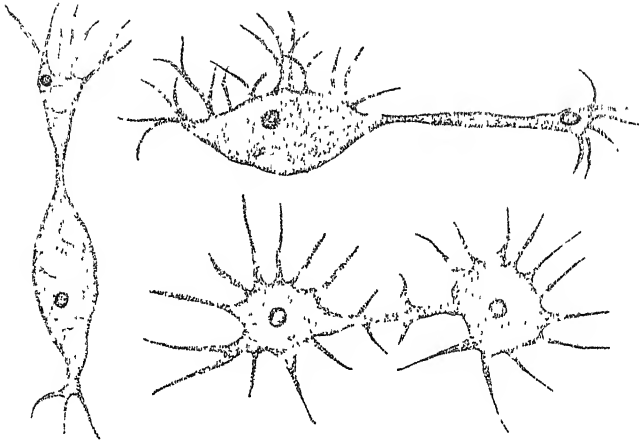


Fig. 2. The fusion of two leucocytes. $\times 1400$.

Agglutinated leucocytes usually lose their proper forms, owing to the mutual pressure. Distinct boundaries of cells, however, were seen in every leucocyte. The distinction of cell identity was easier in the vitally stained cells compared with the colorless cells. The pseudopodia disappeared in the cells which were located in the center of cell-clotting, while the cells which were peripherally did not lose the pseudopodia on their free surfaces.

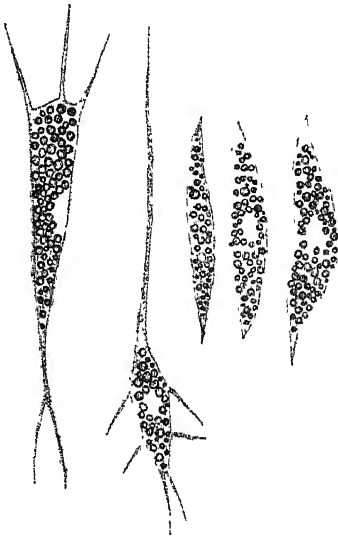


Fig. 3. Fusiform cells with or without pseudopodia. $\times 1400$.

As a result of vital dye-injection, the fusiform cells with or without pseudopodia increased in the coelomic fluid (Fig. 3). These cells might originate chiefly from the epithelium of the coelomic wall or the water vascular system. For the numbers of spindle cells containing dye granules were seen in these portions, and they were just ready to begin proliferation.

The leucocytes and amoebocytes of *Caudina* in vitro have long, spine-like, branching or not branching pseudopodia projecting in all directions. GOODRICH ('19) claims, however, that the leucocytes of the blood or coelomic fluid of the invertebrates *Coelomata* are provided with more or less extensive

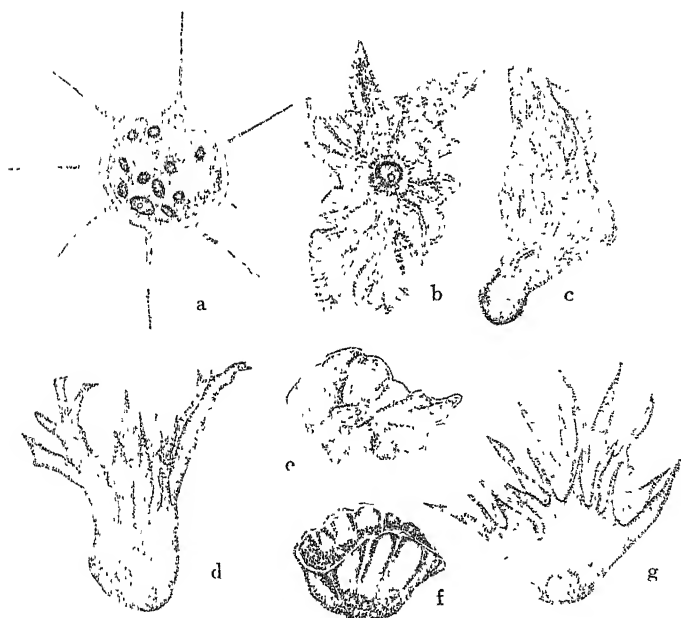


Fig. 4. a Leucocyte in vitro Fine, spine-shaped pseudopodia are seen In the cytoplasm, there are three vacuoles and many dye granules. $\times 1400$.
b — g. Leucocytes after the fixation using GOODRICH's method. $\times 1400$.

membranous processes of cytoplasm. According to him the freely projecting pseudopodia usually described are either figured from optical sections of the folded membranes or from the cells which have produced them under abnormal conditions. Following GOODRICH's method of fixation (a strong solution of iodine in potassium iodide may be used) one is able to detect the presence of membranous pseudopodia in the leucocytes and amoebocytes of *Caudina* (Fig. 4).

In addition to the leucocytes and amoebocytes with colorless spherules, the amoebocytes with brown spherules and minute corpuscles showed occasionally the positive reaction to the vital staining, but the number of dye granules was relatively few in these corpuscles (Fig. 5).

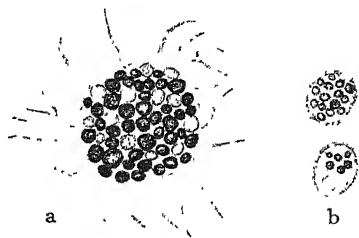


Fig. 5. a Amoebocyte with brown spherules and vital-dye granules $\times 1400$.

b. Minute corpuscles with vital-dye granules. $\times 1400$.

SUMMARY

1. The reaction of coelomic corpuscles of *Caudina chilensis* to trypan blue and carmine was examined. The white, fusiform, brown, and minute corpuscles ingested these dyes abundantly or sparsely, while red, and crystal corpuscles contained none of them.

2. The white corpuscles may be divided into two groups: the leucocytes and amoebocytes with colorless spherules. The former show active vital-dye ingestion while the latter none or less active.

3. The coelomic corpuscles tend to agglutinate in drawn perivisceral fluid. The cells forming the clotting are chiefly leucocytes.

4. As a result of the vital staining, the number of fusiform cells in the perivisceral fluid was increased.

LITERATURE CITED

- GOODRICH, E. S., 1919. Pseudopodia of the leucocytes of invertebrates. *Quart. Jour. Micr. Sci.*, Vol. 64, pp. 19-27.
- KAWAMOTO, N., 1927. The anatomy of *Caudina chilensis* (J. MÜLLER) with especial reference to the perivisceral cavity, the blood and the water vascular system in their relation to the blood circulation. *Sci. Rep. Tôhoku Imp. Univ. Biol.*, Vol. 2, pp. 239-264.
- KINDRED, J. E., 1921. Phagocytosis and clotting in the perivisceral fluid of *Arbacia*. *Biol. Bull.*, Vol. 50, pp. 144-152.
- 1926. A study of the genetic relationships of the "amoebocytes with spherules" in *Arbacia*. *Ibid.*, Vol. 50, pp. 147-154.
- THÉEL, H., 1920-1921. On amoebocytes and other coelomic corpuscles in the perivisceral cavity of echinoderms. *Ark. för. zool.*, Bd. 13, pp. 1-40.

ON THE COELOMIC CORPUSCLES IN THE BODY FLUID OF SOME INVERTEBRATES

II. ON THE COELOMIC CORPUSCLES OF AN EARTH WORM, *DRAWIDA HATTAMIMIZU*, HATAI

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(With Plate II and 1 text-figures)

(Received March 31, 1934)

HATAI ('30) found a new species of earth worm, *Drawida hattamimizu*, recently. I set about the histological study on the body fluid of this earth worm, under the kind suggestion of Prof. HATAI. I should like to express my indebtedness to him for his interest in this work.

The microscopic observations of the body fluid of the oligochaetes have been made by ROLLESTON (1877), CUÉNOT (1891), KENG (1895), ROSA (1896), BENHAM ('01), KOLLMANN ('08) and some other investigators, but so far as I am aware no complete report has appeared on the leucocytes of worms belonging to Moniligastridae.

MATERIAL AND METHODS

Drawida hattamimizu were collected exclusively from Hattamura, a village near Kahoku lake in the prefecture of Ishikawa. The first observation was made of coelomic corpuscles in vitro in a hanging drop of body fluid. For the reaction of the coelomic corpuscles to vital stains, trypan blue and carmine were used. The preparation of these dye-solutions followed the processes which have been described in the fore-going paper. Intraperitoneally the dosage of trypan blue for worms is generally 0.2 c.c. of 0.1 per cent solution per 10 g. body weight. The injection of a larger amount frequently causes the splitting of worm body from the portion of administration. The tolerated dose of carmine is 0.2 c.c. of 0.5 per cent solution per 10 g. body weight. For the study of stained smears, MAY-GIEMSA's and WRIGHT's methods were employed.

OBSERVATIONS

Five types of coelomic corpuscles are present in the body fluid of *Drawida hattamimizu*: a) Lymphocytes; b) Monocytes; c) Granulocytes; d) Lamprocytes; and e) Linocytes.

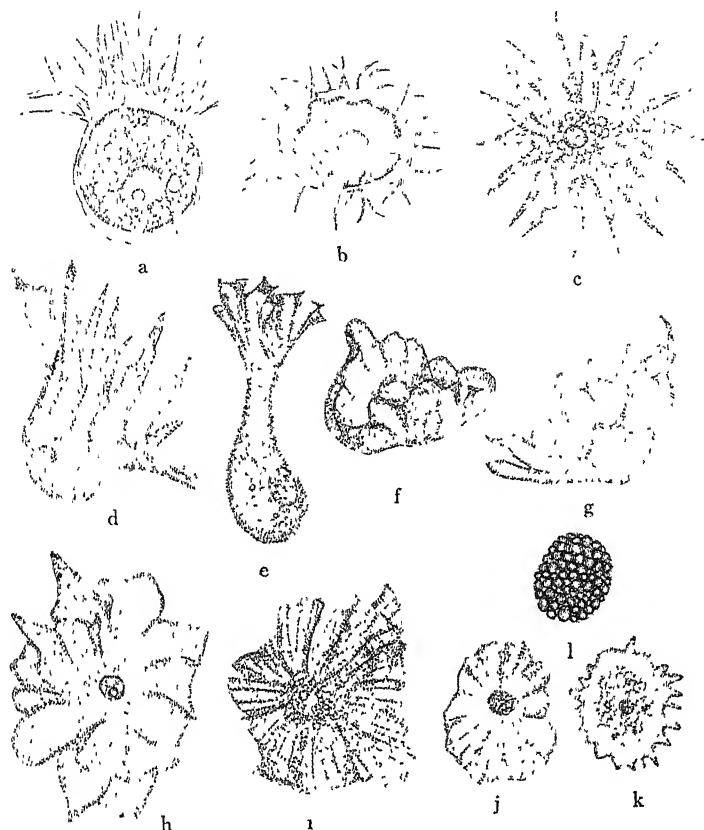
a) Lymphocytes. This kind of leucocytes is most abundant in the body fluid, and they constitute about 53 per cent of total leucocyte count. The lymphocytes in vitro are minute spherical cells with fine pseudopodia, measuring about 4 to 8 micra in diameter. The nucleus is ovoid and coarsely granular. The ratio of nuclear mass to cytoplasmic mass is rather low when compared with the lymphocytes of the vertebrates. Single nucleus is usually found at the eccentric position, but two nuclei may be seen upon rare occasions. The cytoplasm is homogeneous and sometimes contains minute vacuoles. The lymphocytes showed active phagocytosis. They contained the granules of trypan blue or carmine in the vital preparations with these dyes (Plate II, Fig. 4-5).

In smears of the body fluid, stained with either MAY-GIEMSA's or WRIGHT's stain, the lymphocytes are more or less basophilic (Pl. II, Fig. 1-3). The nucleus is stained reddish purple in color, and deeply basophilic coarse granular substances are visible. In the cytoplasm, more or less stained deep bluish depending upon the degree of flattening of the cells when smeared, clear vacuoles are occasionally present. The lymphocytes showed agglutination into plasmodia in vitro, but the boundaries of every cell distinctly remained. In both the vital and smear preparations such massing of lymphocytes is also frequently found.

b) Monocytes. The monocytes when observed in vitro are apparently of the same nature as regards their cytoplasmic contents and nuclear structure, but the cells are larger and measure about 8-12 micra in diameter. They are actively phagocytic, amoeboid, and tend to agglutinate into plasmoid. In the smears, pseudopodia were frequently well reserved (Pl. II, Fig. 6). The nucleus is large, situated eccentrically, and occupies about half of the total cell mass. The form of nucleus is oval, lobed, or bean-shaped. Trypan blue and carmine used vitally have the same effect upon the cytoplasm of monocytes as they have upon that of lymphocytes (Pl. II, Fig. 8-9). Different count of monocyte is 11 per cent.

The pseudopodia of lymphocytes and monocytes are long and spine-shaped, and projecting in one side or in all directions, when the body fluid is examined in vitro (Text-fig. 1, a, b). After GOODRICH's method of fixation, which I have cited in the fore-going paper, the pseudopodia are membranous or petaloid (Text-fig. 1, c — k).

In MAY-GIEMSA's or WRIGHT's stain, the nucleus is basophilic and of coarse granules. The cytoplasm is also basophilic, but occasionally the acidophilic, minute granules, scattered in the inner zone of cytoplasm, were observed.



Text Fig 1. a—b. Lymphocytes in vitro. $\times 1400$.

c—k. Lymphocytes after the fixation of GOÖDRICH's method.

Various forms of pseudopodia are seen.

l. Granulocyte in vitro.

c) Granulocytes. In this group of leucocytes two subtypes are distinguished.

1. *Eosinophilic granulocytes*. These cells present in the body fluid of *Drawida* very abundantly, and they constitute about 32 per cent of total leucocyte count. Such high percentage of eosinophils may be due to the infection of a gregarine, *Monocystis* sp. which is found very abundantly in the intestine of the worm. The size of eosinophils varies remarkably. A great majority of eosinophils are of the same size or two to three times larger than the monocytes, but some of them are as small as the lymphocytes (Pl. II, Fig. 10–12). Between these two extremes there are all degrees of size. The nucleus is small, and more spherical and coarser

than that of lymphocytes. The number of nuclei is usually only one, but there are occasionally the eosinophils which possess two or three nuclei (Pl. II, Fig. 12).

In smears the coarse chromatin granules are stained reddish purple. The cytoplasm is filled with spherical eosinophilic granules. Regarding these granules, the eosinophils are divided into two groups, those with fine granules and those with coarse granules. The former are less numerous than the latter. In a given cell, however, these granules are usually almost the same size. The eosinophils are exclusively negative to the vital stains, but show slow amoeboid movement.

2. *Basophilic granulocytes*. In the body fluid of *Drawida*, these cells are difficult to find because they form only about $\frac{1}{2}$ to 1 per cent of the total number of leucocytes. They measure about 10 micra in diameter. The nucleus is oval or spherical in form, with eccentric location, and usually hidden by the granules (Pl. II, Fig. 13-15). The chromatin is as coarse and pale as those of the eosinophils. The granules in the protoplasm of the living cells are highly refringent. In smears, the granules are intensely stained with basophilic dyes, and show the metachromaticity. No granule of trypan blue or carmine was observed in the basophilic granulocytes drawn from worms injected with vital dyes.

d) *Lamprocytes*. The lamprocytes are one of the characteristic elements of the body fluid of the earth worm. The size of lamprocytes varies; all transitions occur from the size of a monocyte to the cells whose diameter is two or three times larger than the monocyte (Pl. II, Fig. 16-18). Their form is spherical or often flattened. They possess usually a single nucleus, but occasionally two or more. The nucleus is relatively small, regularly round, oval or bean-shaped, and has an eccentric position. The lamprocytes are surrounded by a pellicle and do not form pseudopodia. The cytoplasm is filled with a number of closely arranged vacuoles. The number of lamprocytes in the body fluid varies from 1 to 4 per cent of the total leucocyte count.

In smears stained with MAY-GIEMSA's or WRIGHT's stain the cytoplasm of lamprocytes has a faintly metachromatic tinctorial reaction. The nucleus is sometimes pycnotic.

e) *Linocytes*. This type of leucocytes is found also very sparsely in the body fluid of worms. They are cells of about the size of the lamprocytes, but may be larger or smaller, depending upon the stage of development. They are filled with either a single large vacuole or a number of irregularly sized vacuoles in the cytoplasm (Pl. II, Fig. 19-22). GOODRICH

(1896), BENHAM ('01), KINDRED ('29) and other authors reported the presence of a coiled thread in the vacuoles of linocytes, but I failed to detect such a content in any leucocytes of *Drawida*. BENHAM ('01) made detailed observations upon the development of linocytes of some acanthodrilids. According to his description the linocyte is at first spherical, colorless, non-amoeboïd cell, filled with cytoplasm only. A little afterwards numerous vacuoles become visible in the cytoplasm. These vacuoles gradually increase in size by the union of smaller vacuoles with one another. Then the outline of the united vacuoles become refringent and forms a circular ring, and from this ring fine threads are produced. I was able to find occasionally the presence of refringent rings at the marginal portion of large vacuoles. And from this fact it is probable that the linocytes on which I made observation were young ones. In the linocytes which have single large vacuole the nucleus is pushed to one side.

In smears stained with MAY-GIEMSA's or WRIGHT's stain there could be seen the linocyte with large irregularly shaped vacuoles which show faintly acidophilic reaction (Pl. II, Fig. 20-21). The nucleus is spherical or oval, and deeply basophilic. The cytoplasm is also more or less basophilic. It is curious to me that some of linocytes showed positive reaction to vital staining with trypan blue while they are non-amoeboïd cells (Pl. II, Fig. 22).

f) Other cells free in the perivisceral fluid. In addition to the leucocytes, the perivisceral fluid contains detached chloragocytes and peritoneal cells. The chloragocytes are easily distinguished from the leucocytes by their content of brownish yellow globules (Pl. II, Fig. 23).

The parietal peritoneal cells are elongated and spindle-shaped in shape, and have usually several free projecting pseudopodia, chiefly arising from both ends of the cell body. In smears, the nucleus is oval, granular, and basophilic. The cytoplasm is also slightly basophilic and metachromatic. The peritoneal cells show positive reaction to the vital staining with trypan blue or carmine.

COMPARISON OF THE CELLS WITH THOSE OF OTHER OLIGOCHAETES

The knowledge of the leucocytes of the oligochaetes has shown great advance in this century. In his monograph, BEDDARD (1895) states that in the higher oligochaetes the corpuscles are apparently of two kinds: amoeboid cells and large spherical cells loaded with granules. STEPHENSON ('30) gives also information as to the formed constituents of the body fluid

in his monograph. He enumerates seven kinds of leucocytes: amoebocytes, vacuolar lymphocytes, eleocytes, nemerocytes, linocytes, lamprocytes, and mucocytes. Leading contributors of this field are ROSA, BENHAM, CUÉNOT, KOLLMANN, JOSEPH ('09) and so forth. Recently KINDRED ('29) published a report on the leucocytes and leucocytopoietic organs of *Pheretima indica*. The result of the present investigation bears close resemblance to that of KINDRED in general. He found five types of leucocytes in the body fluid of *Pheretima indica*: lymphocytes, monocytes, granulocytes, lamprocytes, and linocytes. Just the same kinds of leucocytes were also found in the present investigation. In the case of *Pheretima indica*, the count of granulocytes is surpassed by the count of monocytes, while in the case of *Drawida* the relation is just the reverse. KINDRED stated that some granulocytes of *P. indica* contain granules with a metachromatic purple tinctorial reaction, and he interpreted these granules as stages in the development of eosinophilic granules. I failed to detect the presence of such transitional granulocytes.

In his report, HATAI stated that the original home land of *D. hattamimizu* might be either India, Java, or the Philippine islands. The localities of *P. indica* are also India, Java and so forth. Therefore both species correspond in that they belong to tropical or subtropical earth worms. Is it unreasonable to suppose that the close resemblance of the leucocytes in these species is due to the close relation of their original home land?

SUMMARY

1. The body fluid of *Drawida hattamimizu* contains five types of leucocytes: the lymphocytes, the monocytes, the granulocytes, the lamprocytes, and the linocytes. In addition to the leucocytes the perivisceral fluid contains detached chloragocytes and peritoneal cells.

2. The lymphocytes, monocytes and peritoneal cells show the positive reaction to the vital staining with trypan blue and carmine. The vital preparation of linocytes contained also granules of trypan blue upon rare occasions.

3. The predominance of eosinophilic granulocytes may be enumerated as a characteristic feature of blood picture of *D. hattamimizu*. They constitute about 32 per cent of total leucocyte count.

4. The experiment failed to detect the thread-like substance in the cytoplasm of linocytes.

LITERATURE CITED

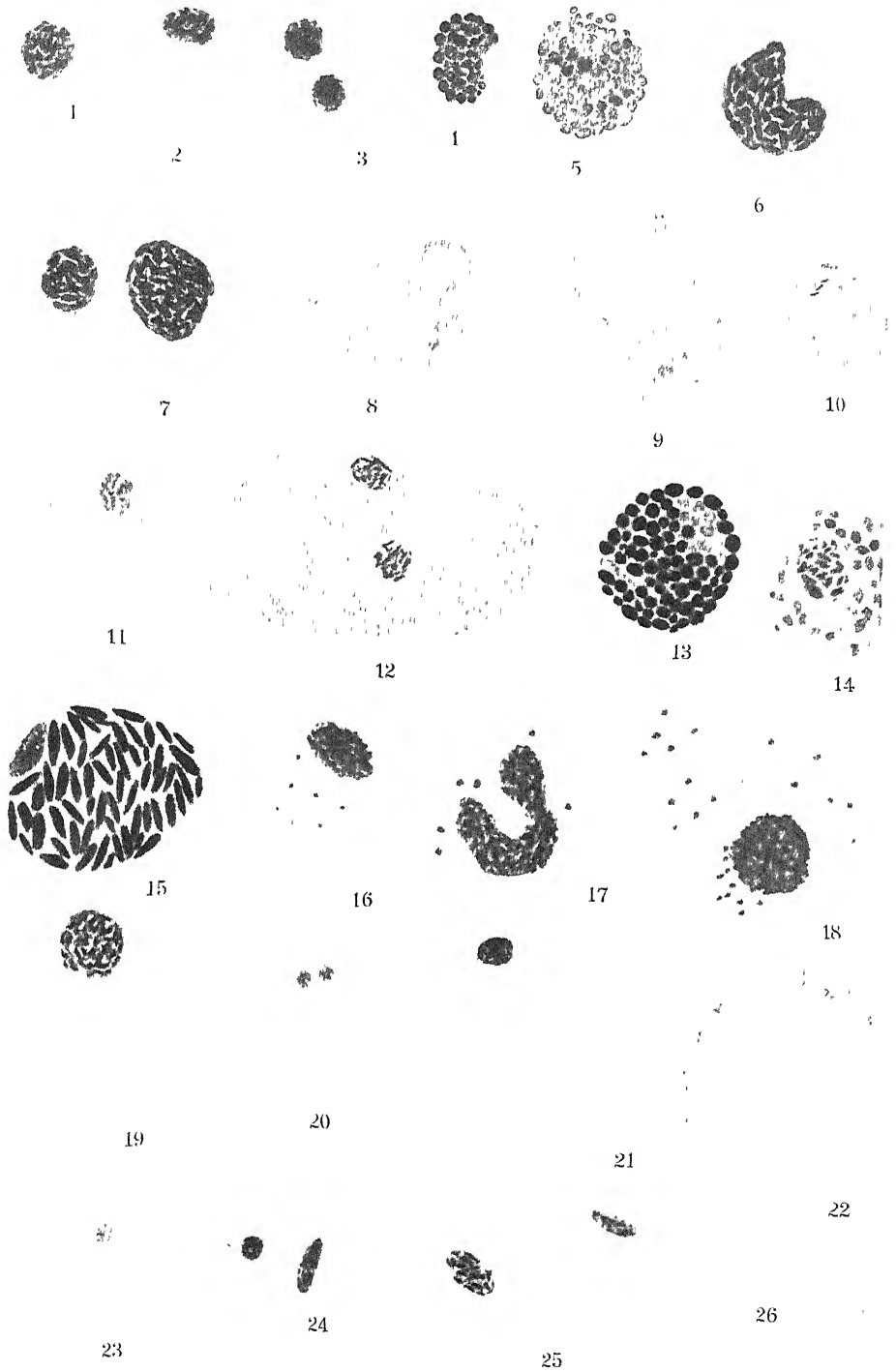
- BEDDARD, F E, 1895. Monograph of the oligochaeta. Oxford.
- BENHAM, W. B., 1901 The coelomic fluid of Acanthodrilids. Quart. Jour. Micr. Sci., Vol. 44, pp. 565-589.
- CUÉNOT, L., 1891 Etudes sur la sang et glands lymphatiques dans la serie animale Partie 2, Invertébrés Arch Zool., Sér 2, T. 9, pp 613-641
- GOODRICH, E S, 1896. Note on the oligochaetes with the description of new species. Quart. Jour. Micr Sci., Vol. 39 pp. 51-72.
- 1919. Pseudopodia of the leucocyte of invertebrates Ibid, Vol. 64, pp. 19-27.
- HATAI, S., 1930. On *Drawida hattamizu*. sp. nov. Sci. Rep. Tohoku Imp. Univ Biol, Vol. 5, pp 485-508.
- JOSEPH, H, 1909. Die amœbocyten von Lumbricus. Arb. Zool. Inst Univ. Wien. Bd. 18.
- KENG, L M., 1095 On the coelomic fluid of Lumbricus terrestris in reference to a protective mechanism. Phil Trans. Roy Soc. London Ser. II B, Vol 186, p. 383.
- KINDRED, J E., 1929. The leucocytes and leucocytopoietic organs of an oligochaete *Pheretima indica* (HORST) Jour. Morph. Physiol, Vol 47, pp. 437-477.
- KOLLMANN, M., 1908. Recherches sur les leucocytes et le tissu lymphoïde des Invertébrés. Ann. Sci. Nat Zool., T. 8, pp. 1-240.
- ROLLESTON, G, 1877. The blood corpuscles of the annelides. Jour. Anat. Physiol, Vol. 12, pp. 401-418.
- ROSA, D, 1896. Les lymphocytes des oligochètes Arch. Ital. de Biol, T. 25, pp. 455-458.
- STEPHENSON, J, 1930 The oligochaeta Oxford

PLATE II

DESCRIPTION OF FIGURES

All of the figures illustrating this plate were drawn with the aid of camera lucida. $\times 2000$.

- 1-3 Lymphocytes from smears of perivisceral fluid. MAY-GIEMSA.
- 4-5 Lymphocytes in vitro, vitally stained with trypan blue.
- 6-7 Monocytes. Smear. MAY-GIEMSA.
- 8-9 Monocytes in vitro, vitally stained with trypan blue.
- 10-12 Three types of eosinophilic granulocytes. Smear. MAY-GIEMSA.
- 13-15 Three types of basophilic granulocytes. Smear. MAY-GIEMSA
- 16-18 Lamprocytes. Smear. MAY-GIEMSA.
- 19 Linocytes with many vacuoles. Smear MAY-GIEMSA.
- 20-21 Linocyte with large single vacuole. Smear MAY-GIEMSA.
- 22 Linocyte in vitro. Vitally stained with trypan blue
- 23 Chloragocyte. Smear. GIEMSA
- 24-26 Parietal peritoneal cells. Smear. MAY-GIEMSA.



CONTRIBUTIONS TO THE PHYSIOLOGY OF THE ASCARIS

II. THE RESPIRATORY EXCHANGE IN THE ASCARIS, *ASCARIS MEGALOCEPHALA* CLOQ.

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(With 3 text-figures)

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INTRODUCTION

It is generally thought that the animals which normally exist in the absence of oxygen consume the glycogen stored in their bodies for certain manifestation of life, giving as a product carbon dioxide. *Ascaris* is, therefore, very instructive for investigation of anaerobic respiration because of the fact that they live in the small intestine, where oxygen is almost absent and furthermore they contain a large quantity of glycogen in their bodies as has already been stated by WEINLAND (1901-1906) in *Ascaris lumbricoides* and also by the present author (1932) in *Ascaris megalocephala*.

The usual seat of ascaris in host is the small intestine, but they often wander into the stomach, and exceptionally get into the bronchi, nose, coelom, etc., where oxygen is present. *Ascaris* may be, therefore, a worm of facultative anaerobic existence, accordingly the respiratory exchange of ascaris in presence of oxygen is also of interest.

The most recent work on the respiratory exchange of the ascaris is that of WEINLAND (1901-1906). He found that *Ascaris lumbricoides* outputs 0.4 g. of carbon dioxide in twenty-four hours in the absence of oxygen. As far as I know, however, there is no one who has studied the respiratory exchange in *Ascaris megalocephala* in the absence of oxygen, still less in the presence of it.

In the present work I have dealt with the respiratory exchange in the absence of oxygen from a healthy condition to the point of death, as the second step of investigations concerning the anaerobic changes of the ascaris, with the hope of obtaining further data about the metabolism of the glycogen stored in the worm, and also with the respiratory exchange in presence of oxygen to determine the question as to whether the oxygen consumption is possible or not.

MATERIAL AND METHOD

The ascarides used in my experiment were found in the small intestines of horses raised for anatomical researches of the students of the Morioka Imperial College of Agriculture and Forestry. Some of the specimens were also collected from the Morioka Slaughter House. In this experiment only fresh, healthy specimens varying in weight from about 2 to 7 gms. in the female worms and from 1 to 2 gms. in the male ones, were used.

For the determining of the amount of carbon dioxide produced in the anaerobic existence of the worm, the following medium was used; RINGER's solution was boiled in order to drive off the oxygen and then saturated with nitrogen that was purified by passing it through a pyrogallie acid solution. Thus the solution contained no oxygen.

For the determining not only the amount of carbon dioxide produced, but also the oxygen consumed in the aerobic existence of the worm, RINGER's solution of a known concentration of oxygen and carbon dioxide was used.

Preceding the experiment each specimen was kept in the solution for several hours at the temperature to which it was to be subjected. The specimens were placed separately in a glass bottle containing 500 cc. of the solution above mentioned. In order to prevent direct contact with the air, the top of the solution was covered with paraffin oil about 5 cm. thick, as is usual in such experiments. It was not necessary to stir the medium during the experiments, because a uniform distribution of the gases in the medium was facilitated by the continuous peristaltic movement of the worm. By placing the bottle in a thermostat the temperature was kept constant at 38°C. which is the normal body temperature of the horse. Under these conditions the worm lived for about 25 to 40 hours.

To determine the decrease and the increase of the oxygen and the carbon dioxide in the medium, caused by the respiration of the worm, VAN SLYKE's method was used. In my experiment, 2 cc. of medium at fixed intervals were used for analysis.

1 N. lactic acid was used for freeing the carbon dioxide from the medium which contain carbonate.

The reagent used for the absorption of the oxygen was alkaline pyrogallol, while for the absorption of the carbon dioxide a 5 N. sodium hydroxide solution was used.

RESULT OF THE EXPERIMENT

Preceding the experiment on the respiratory exchange of *Ascaris megalocephala* CLOQ., I have analysed the gases of the intestinal fluid of the horse, with the following result.

	Total CO ₂ in Vol. %	Free CO ₂ in Vol. %	CO ₂ as carbon- ates in Vol. %	O ₂ in Vol. %
Duodenum	68.515	6.334	62.189	0.031
Jejunum	70.520	8.167	62.351	0.016

As will be seen from the above table, the total amount of carbon dioxide contained in the intestinal fluid of the horse is about 70 per cent, 7 per cent being free carbon dioxide and 63 per cent is combined as carbonates, while oxygen is almost absent, being only about 0.02 per cent. *Ascaris megalocephala* is, therefore, anaerobic in its usual seat.

1. *Results Obtained for the Respiratory Exchange in
Absence of Oxygen*

The full test of each experiment is not given, but I may add that the range of variation was very small throughout the entire series of experiments. I have given the numerical data of three cases only, the first representing the adult females, the second the young females and the third the males, in Table I, and that of others are shown only by curves.

TABLE I
Carbon dioxide evolved in absence of oxygen

Body weight in gms.	Time in hours	CO ₂ content in Vol. %	CO ₂ evolved in Vol. %
5.20(♀)	0	3.854	
	1	3.907	0.053
	3	3.996	0.142
	5	3.786	0
	7	3.985	0.131
	9	3.852	0
	10	4.360	0.406
	12	4.485	0.631
	14	4.501	0.647
	18	4.577	0.723
	21	4.621	0.767
	23	4.635	0.781

Body weight in gms	Time in hours	CO ₂ content in Vol. %	CO ₂ evolved in Vol. %
2.95(♀)	0	4.013	
	2	4.013	0
	4	4.088	0.075
	7	4.042	0.029
	12	4.058	0.045
	15	4.428	0.415
	23	4.498	0.485
	28	4.483	0.570
	32	4.527	0.614
1.29(♂)	0	5.282	
	2	5.264	0
	5	5.527	0.217
	10	5.330	0.052
	15	5.269	0
	27	5.354	0.072
	33	5.395	0.113

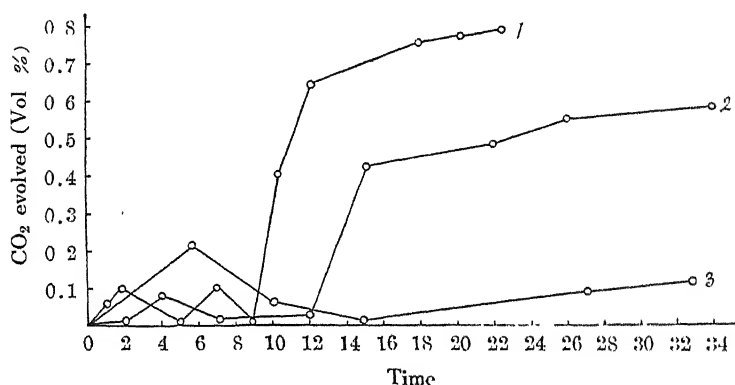


Fig 1. Showing the changes in CO₂ evolved in the medium deprived of oxygen.

1. Adult female (5.20 gms.)

2. Young female (2.95 gms.)

3. Adult male (1.29 gms.)

As is shown in Table I and Figure 1, no carbon dioxide production occurs during the first several hours of the experiment. In about from 9 to 12 hours, however, its production begins. This continues only for a short time; succeeding period shows almost no more production until approaching the point of death. It is also to be noticed in the same table and the figure that the amount of carbon dioxide produced in the large female is greater than that in the small female and the male, and that the duration from the beginning of the experiment to the point at which the carbon dioxide production rises is shorter in the former than in the latter.

From the result above obtained, it is noted that the ascaris can live without oxygen and no carbon dioxide production occurs, except the duration of a few hours in the middle of the experiment, showing a true fermentation process. It seems to me that since a great amount of glycogen normally exists in *Ascaris megalocephala*, as already mentioned in a previous paper (1932), fermentation process undergoes, by which the glycogen probably split into some intermediate substances, but at once it would be used up for reduction process, as has been suggested by WEINLAND in *Ascaris lumbricoides*.

As regards the carbon dioxide produced for a time in the course of the experiment, I noticed the following relation: first, as I have already stated in a previous paper (1932), a large amount of glycogen which is supposed to be used up for active growth of the developing young cells is stored in the reproductive organ of the large female ascaris, but only a small amount of it is stored in that of the small female and the male ascaris, accordingly more carbon dioxide would be derived from the glycogen in the former than in the latter; second, since in about from 9 to 12 hours of the experiment, when the carbon dioxide production begins, RINGER's solution used very likely becomes acidic (pH 6.0-6.5), suggesting the intermediate substances, such as lactic acid or valerianic acid, are formed from carbohydrates in the tissues of the worm, as is usual in anaerobic changes, it is highly probable that the carbon dioxide would be produced from the carbonate which may exist in the worm, and the larger the worm, the more carbonate would be decomposed owing to its greater amount. To support the view just stated I notice in Table I and Figure 1 that the amount of carbon dioxide produced is greater in the large female than in the small female and the male.

2. Results Obtained for the Respiratory Exchange in Presence of Oxygen

The results obtained at 38°C. are given in Table II, and are shown graphically in Figures 2 and 3. In Table III are given the results obtained at room temperature (16°-19°C.).

As will be seen in Table II and Figures 2 and 3, no oxygen consumption nor carbon dioxide production occurs during the first several hours of the experiment. In about from 4 (female) to 20 hours (male), however, the oxygen consumption begins. This continues at almost the same rate during several hours afterwards until the oxygen tension in the medium

TABLE II

Carbon dioxide evolved and oxygen consumed in presence of oxygen

Body weight in gms.	Time in hours	O ₂ content in Vol %	O ₂ consumed in Vol %	CO ₂ content in Vol %	CO ₂ evolved in Vol %	Respiratory quotient
7.22 (♀)	0	0.486		5.327		
	1	0.456	0.030	5.321	0	
	2	0.486	0	5.359	0.032	
	3	0.487	0	5.301	0	
	5	0.473	0.013	5.372	0.015	3.160
	9	0.321	0.165	5.450	0.123	0.745
	13	0.218	0.268	5.583	0.256	0.955
	15	0.093	0.392	6.573	1.216	3.178
	18	0.046	0.439	6.554	1.227	2.393
	22	0.062	0.421	6.580	1.253	2.861
	26	0.059	0.427	6.515	1.278	2.852
	28	0.031	0.455	6.720	1.375	3.022
2.26 (♀)	0	0.513		5.444		
	2	0.513	0	5.465	0.021	
	3	0.512	0	5.380	0	
	5	0.513	0	5.444	0	
	8	0.513	0	5.441	0	
	12	0.530	0	5.181	0	
	20	0.437	0.076	5.165	0.012	0.276
	22	0.312	0.101	5.478	0.031	0.337
	25	0.091	0.422	5.807	0.373	0.884
	27	0.091	0.422	5.933	0.489	1.159
	28	0.061	0.452	5.879	0.435	0.962
	32	0.090	0.423	5.933	0.489	1.156
1.05 (♂)	0	0.565		5.243		
	2	0.557	0.008	5.298	0.053	
	3	0.539	0.026	5.171	0	
	8	0.570	0.005	—		
	15	0.483	0.082	5.118	0	
	19	0.480	0.085	5.248	0	
	22	0.211	0.354	5.280	0.037	0.105
	23	0.276	0.289	5.691	0.418	1.619
	24	0.150	0.415	5.654	0.411	0.988
	27	0.015	0.520	5.761	0.521	1.002
	36	0.047	0.518	5.813	0.570	1.120
	42	0.060	0.475	5.829	0.586	1.234

becomes about 0.06, indicating that the rate of the oxygen consumption is independent of the oxygen tension in the medium as in the aerobic animals. It is also to be noted that during the period just mentioned the carbon dioxide production occurs in the same manner as at the experiment in absence of oxygen, though a smaller amount of it is obtained in this case.

There is remarkable variation in the respiratory exchange between the male and the female; the carbon dioxide production is approximately parallel to the oxygen consumption in the male, but not in the female, in which the amount of carbon dioxide produced exceeds that of the oxygen consumed.

TABLE III

Carbon dioxide evolved and oxygen consumed in presence of oxygen (16°-19°C.)

Body weight in gms	Time in hours	O ₂ content in Vol. %	O ₂ consumed in Vol. %	CO ₂ content in Vol. %	CO ₂ evolved in Vol. %	Respiratory quotient
5.10 (♀)	0	0.709		4.728		
	19	0.623	0.086	4.794	0.066	0.767
	43	0.125	0.584	5.556	0.826	1.414
	67	0.031	0.678	5.999	1.271	1.875
	87	0.031	0.678	6.194	1.466	2.162
1.85 (♂)	0	0.623		5.195		
	19	0.436	0.187	5.288	0.093	0.492
	44	0.125	0.498	5.580	0.385	0.853
	66	0.031	0.582	5.732	0.537	0.924
	85	0.031	0.582	5.675	0.480	0.828

As is shown in Table III, a similar relation, as far as the oxygen consumption and the carbon dioxide production concern, is also observed in the experiment at room temperature. In this case, the survival of the worm is twice as much as at 38°C.

The results obtained from the above experiment seem to indicate that

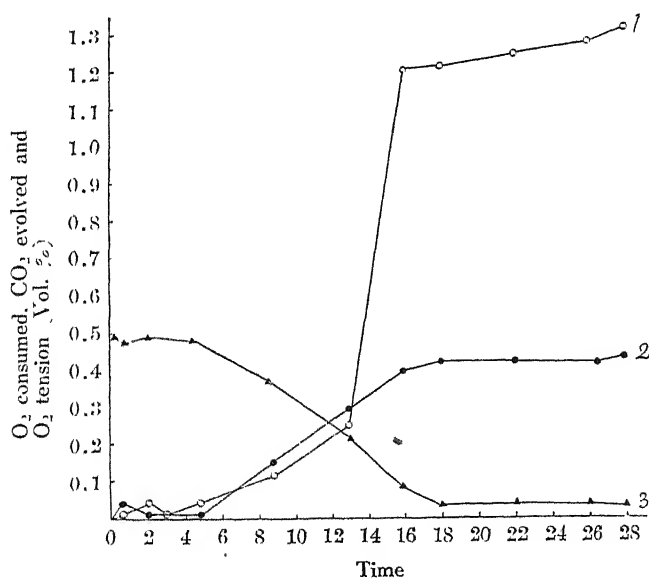


Fig. 2 Showing the changes in O₂ consumed and CO₂ evolved in the medium supplied of oxygen (Adult female).

1 CO₂ evolved. 2. O₂ consumed. 3. O₂ tension.

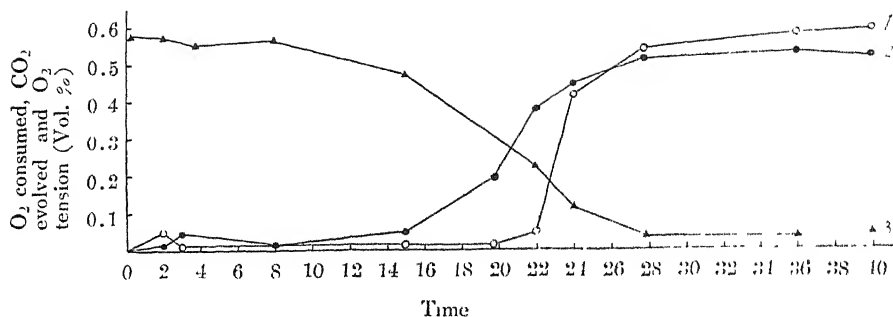


Fig. 3. Showing the changes in O₂ consumed and CO₂ evolved in the medium supplied of oxygen (Adult male)

1. CO₂ evolved 2. O₂ consumed. 3. O₂ tension.

since ascarides normally exist in the absence of oxygen they find it very difficult to take it at first, but soon become adapted to its presence and oxydative reaction takes place. Thus the male perhaps burns up glycogen to carbon dioxide and water in presence of oxygen. To support the view just stated I noticed that the respiratory quotient in the male is about 1, as will be seen in Table II and Figure 3.

A similar oxydative process perhaps occurs in the large female, but from the fact that the carbon dioxide produced exceeds the oxygen consumed (Table II and Fig. 2), I suppose that some chemical process of a special kind without oxygen, by which the carbon dioxide production takes place, occurs in the female. As regards the process in question, it is probably associated with the developing young ovum as well as that in the absence of oxygen. Further work is being undertaken, as to the question just stated.

To compare the respiratory exchange in the presence of oxygen with that in the absence of it, the total amount of oxygen consumed and the carbon dioxide evolved were calculated. The result is given in Table IV. In the same table is also given the data of several other parasites for comparison with that of *Ascaris megalocephala*.

As will be seen in Table IV, the amount of carbon dioxide produced in the presence of oxygen is higher than that in the absence of it, showing the fact that not only a fermentation process, but oxydation occurs in the presence of oxygen. It is also to be noted that the production of carbon dioxide in *Ascaris megalocephala* is much less than that found for *Ascaris lumbricoides* by WEINLAND and for *Filaria equina* by the present author. The variations just stated are probably due to the specificities of

TABLE IV

Parasite	Temperature	Time in hours	Cases	O ₂ consumption per 100 gms. in cc.	CO ₂ output per 100 gms in cc	Respiratory quotient	Investigators
<i>Ascaris megalocephala</i> (♂)	16°-19°C.	41	Presence of oxygen	135	124	0.911	TORYU
" (♀)	"	"	"	57	81	1.432	
" (♂)	38°C	24	"	245	195	0.796	
" (small ♀)	"	"	"	92	68	0.739	
" (large ♀)	"	"	"	29	84	2.896	
" (♂)	"	"	Absence of oxygen		20		
" (small ♀)	"	"	"		58		
" (large ♀)	"	"	"		75		
<i>Ascaris lumbricoides</i>	"	"	"		204		WFINLAND
<i>Filaria equina</i>	"	"	"	625	650	1.040	TORYU

the worm used and technique employed on one hand, and to the natures of their respiration owing to the living place of the worms on the other hand.

SUMMARY

1. *Ascaris megalocephala* is not a worm of obligatory anaerobiosis, but of facultative anaerobiosis; namely they produce carbon dioxide by a fermentation process in the absence of oxygen, and by an oxidative reaction in the presence of it.

2. When the worms are placed in RINGER's solution containing oxygen, they consume the oxygen until the tension in the medium becomes about 0.03.

3. Carbon dioxide production is almost parallel to the oxygen consumption in the male as well as in the young female, but not in the adult female, in which the carbon dioxide produced exceeds the oxygen consumed.

4. When the worms are placed in RINGER's solution deprived of oxygen, they also produce carbon dioxide, though the amount of it is much less than that obtained in the presence of it.

5. The total amount of carbon dioxide produced in twenty-four hours at 38°C. was from 80 (female) to 200 cc. (male) per 100 gms. of the worm

in the presence of oxygen, and from 20 (male) to 80 cc. (female) in the absence of it.

6. Almost no carbon dioxide production nor oxygen consumption occurred during the first and the last several hours of the experiment, suggesting a true fermentation process.

Before leaving the subject, I wish to express my hearty thanks to Dr. S. HATAI, for his valuable suggestions throughout the entire course of this work.

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LITERATURE

- OTTO VON FURTH 1903 Vergleichende chemische Physiologie der niederen Tiere. Jena
- TORYU, Y. 1932 Contributions to the physiology of the ascaris. I Glycogen content of the ascaris, *Ascaris megalocephala* Cloq. Sci. Rep. Tohoku Imp. Univ. Biol., Vol 8, No. 1, pp 65-74.
- WEINLAND, E. 1901 Über Kohlenhydratzersetzung ohne Sauerstoffaufnahme bei *Ascaris*, einen tierischen Gärungsprozess. Zeitschr. f. Biol., Bd. 12 pp. 55-90.
- WEINLAND, E. 1906 Über ausgepreste Extrakte von *Ascaris lumbricoides* und ihre Wirkung. Zeitschr. f. Biol., Bd. 43 pp. 89-111.

ON COMPENSATION POINT OF WOODY PLANTS¹⁾

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INTRODUCTION

The compensation point, the minimum light intensity at which the CO_2 produced by respiration equaled that consumed in photosynthesis, is generally studied in the course of the investigations of the assimilation of carbon dioxide. PLAETZER (1917) studied the compensation point by water plants and recently ERKE (1931) investigated in this line with sea-algae. For the land plants it was also studied by many investigators such as JOHANSSON (1923), LUNDEGÅRDH (1921, 1924), STÅLFELT (1921) and BOYSEN JENSEN (1918, 1932) and they arrived at the same conclusion that the compensation point by sun-plants took place always under a light intensity stronger than the shade-plants. These investigators treated mainly the typical sun- and shade-plants and left almost untouched the study with woody plants. BURNS (1923) is the only one, so far as I am aware, who studied many woody plants in America. In our country the studies along this line are quite deficient. To contribute some knowledge, I studied the compensation point with 70 woody plants which grow mostly in the northern part of our country.

. METHOD

The experiment was carried out in a room of constant temperature at 25°C. The air was lead from the outside of the room to the assimilation chamber through a long snaked tube. A cubic glass vessel of a content of ca. 100 c.c. was used as the assimilation chamber. The snaked glass tube was placed in the room of constant temperature so that the temperature of the air was held constant through the experiments.

A Mazda lamp of 300 watts was used as the light source. The light, reflected by a paraboloidal reflector of enamelled tin, illuminated the assimilation chamber rectangularly. The light intensity is determined by

¹⁾Contributions from the Mt Hakkôda Botanical Laboratory. No. 20.

a foot candle meter¹⁾ at the place, where the compensation point was estimated.

For the experiments a leaf, a leaflet or a small branch with many scale leaves was used. They were gathered from the garden and immediately brought to the room having a constant temperature and then prepared for the assimilation. The material was exposed for 12 minutes for the experiment. The distance from the light source of the assimilation chamber was changed for each experiment. The absorption of carbon dioxide in the air which passed through the assimilation chamber during the illumination was determined by the method of BOYSEN JENSEN and MULLER (1928). When neither increase nor decrease of carbon dioxide in the air was found, the light intensity at this point was measured. Thus the compensation point was obtained.

The experiments were carried out during the growing season in 1931. To ascertain the results obtained in the year 1931 the same experiments were repeated with the same species in the following year. The difference of the light values of compensation point in both cases was not remarkable, namely it fluctuated within 2 per cent. in general.

It is generally referred that the intensity of carbon assimilation in the field is not the same in the morning and in the afternoon. But this was not ascertained for the compensation point so far as my experiments are concerned. Some experiments were carried out, therefore, in the afternoon, although most of them were made in the morning.

RESULTS

According to their life forms the plants investigated are divided into the following four groups, namely, 1) summer-green plants, 2) needle plants, 3) broad-leaved ever-green plants and 4) shade-plants. The plants of the first three groups belong to the sun-plant.

1) Summer-green plants (Table 1).

As shown in the table (Table 1), the light values of compensation point of leaves of this group show the most remarkable variation in these four groups, and are large enough as a whole and the light intensity for the compensation point was proved in about 50 per cent. of the plants between 1000 and 1200 m.c. (abbr. for meter candles). *Corylopsis pauciflora*, *C. spicata*, *Quercus crispula*, *Zelkova serrata*, *Hydrangea paniculata* var.

¹⁾For the comparison of the value, the energy of the electric lamp was also measured by a thermopile. For instance the light intensities of 1360 and 440 meter candles correspond to 0.0100 and 0.0157 g cal. per minute respectively.

TABLE 1.

<i>Rhus javanica</i> L.	2010 m.e
<i>Cornus Kousa</i> Buerger.	1760
<i>Corylopsis pauciflora</i> Sieb. et Zucc.	1400
<i>C. spicata</i> Sieb. et Zucc.	1370
<i>Quercus crispula</i> Blume	1360
<i>Zelkova serrata</i> Makino	1360
<i>Hydrangea paniculata</i> Sieb. var. <i>floribunda</i> Regel.	1320
<i>Fraxinus Sieboldiana</i> Blume var. <i>serrata</i> Nakai	1320
<i>Carpinus laxiflora</i> Blume	1300
<i>Micromela alnifolia</i> Kochne	1300
<i>Ulmus parvifolia</i> Jacq.	1270
<i>Corylus heterophylla</i> Fisch. var. <i>japonica</i> Koidz.	1250
<i>Castanea crenata</i> Sieb. et Zucc.	1250
<i>Rhodotypos scandens</i> Makino	1250
<i>Quercus serrata</i> Thunb.	1190
<i>Acer cissifolium</i> C. Koch	1160
<i>Edgeworthia papyrifera</i> Sieb. et Zucc.	1150
<i>Lagerstroemia indica</i> L.	1150
<i>Quercus acutissima</i> Carr.	1110
<i>Lonicera gracilipes</i> Miq. var. <i>glabra</i> Miq.	1130
<i>Betula latifolia</i> Kom.	1120
<i>Vaneria tricuspidata</i> Hu	1120
<i>Amelanchier asiatica</i> Endl.	1120
<i>Celtis sinensis</i> Pers.	1100
<i>Parabenzoin praecox</i> Nakai	1100
<i>Idesia polycarpa</i> Maxim.	1100
<i>Quercus variabilis</i> Blume	1060
<i>Alangium platyfolium</i> Harms. var. <i>macrophyllum</i> Wang.	1010
<i>Acer pictum</i> Thunb. var. <i>typicum</i> Graf v. Schw. subvar. <i>eupictum</i> Pax.	1020
<i>Hanamelis japonica</i> Sieb. et Zucc.	1010
<i>Prunus Grayana</i> Maxim.	1010
<i>Stachyurus praecox</i> Sieb. et Zucc.	1010
<i>Populus Sieboldii</i> Miq.	1000
<i>Styrax japonicum</i> Sieb. et Zucc.	1000
<i>Phellodendron amurense</i> Rupr.	950
<i>Clethra barbinervis</i> Sieb. et Zucc.	950

<i>Cornus contrivasa</i> Hemsl.	870
<i>Ginkgo biloba</i> L.	740
<i>Magnolia praecocissima</i> Koidz.	660
Mean value of light intensity	1110

floribunda and *Fraxinus Sieboldiana* var. *seriata* require strong light intensity over 1300 m.c. and especially *Rhus javanica* and *Cornus Kousa* require a light intensity exceeding 1500 m.c.

On the contrary the light value of compensation point of *Phellodendron amurense*, *Crethra barbiniensis*, *Cornus contrivasa*, *Ginkgo biloba* and *Magnolia praecocissima* is under 1000 m.c.. The value is low as these plants are the sun-plant. The light value of *Ginkgo biloba* and *Magnolia praecocissima* is especially low and nearly one-third of that of *Rhus javanica* and near a half of most other plants. This value approaches to that of the shade-plant which will be described below.

2) Needle plants (Table 2).

The value of the light intensity at the compensation point of these plants is generally higher (mean value of 1180 m.c.) than that of the

TABLE 2.

<i>Thuja orientalis</i> L.	1520 m.c.
<i>Pinus densiflora</i> Sieb. et Zucc.	1300
<i>Taxus cuspidata</i> Sieb. et Zucc.	1250
<i>Chamaecyparis obtusa</i> Sieb. et Zucc. var. <i>breviramea</i> Mast.	1250
<i>Abies firma</i> Sieb. et Zucc.	1230
<i>Juniperus chinensis</i> L.	1230
<i>Torreya nucifera</i> Sieb. et Zucc.	1210
<i>Chamaecyparis pisifera</i> Sieb. et Zucc. var. <i>squarrosa</i> Mast.	1210
<i>Sciadopitys verticillata</i> Sieb. et Zucc.	1170
<i>Chamaecyparis pisifera</i> Sieb. et Zucc. var. <i>plumosa</i> Mast.	1110
<i>C. pisifera</i> Sieb. et Zucc.	1080
<i>Thuyopsis dolabrata</i> Sieb. et Zucc.	1050
<i>Larix Kaempferi</i> Sarg.	1010
<i>Chamaecyparis obtusa</i> Sieb. et Zucc.	1030
<i>Cryptomeria japonica</i> D. Don	960
Mean value of light intensity	1180

summer-green plants (mean value of 1140 m.c.). No plant, however, in this group has such a high value as *Rhus javanica*, although *Thuja orientalis* and *Pinus densiflora* require quite intense light for the minimal assi-

milation. But *Thuopsis dolabrata*, *Larix Kaempferi*, *Chamaecyparis obtusa* and *Cryptomeria japonica* could assimilate in the light intensity near 1000 m.c.. There is no plant in this group with such an excessive low value as *Ginkgo biloba* and *Magnolia praecocissima*.

3) *Broad-leaved ever-green plants* (Table 3).

This group of broad-leaved ever-green plants has a higher compensation point than the former groups and the mean value of light intensity reaches

TABLE 3.

<i>Ilex crenata</i> Thunb. var. <i>typica</i> Loes f. <i>genuina</i> Loes	1580 m.c.
<i>Daphniphyllum macropodum</i> Miq.	1430
<i>Eurya japonica</i> Thunb.	1420
<i>Quercus myrsinaefolia</i> Blume	1310
<i>Camellia japonica</i> L. var. <i>hortensis</i> Makino	1210
<i>Ligustrum japonicum</i> Thunb.	1200
<i>Trochodendron aralioides</i> Sieb. et Zucc.	1190
<i>Quercus phyllinaeoides</i> A. Gray	1170
<i>Osmanthus ilicifolius</i> Standish	1150
<i>Tetradenia glauca</i> Matsum.	1120
<i>Ilex latifolia</i> Thunb.	1100
Mean value of light intensity	1235

as high as 1235 m.c. and no plant could endeavour under the light intensity of 1100 m.c. The light value of *Ilex crenata* var. *typica* f. *genuina* is the highest.

It is noteworthy that no remarkable difference of the compensation point between the needle plants and the broad-leaved ever-green plants was obtained, although they are quite different in anatomical structure of leaf. In this case it is assumed that at least in the minimal light intensity the leaf structure will not decide the difference of CO₂-assimilation.

4) *Shade-plants* (Table 4).

TABLE 4.

<i>Fatsia japonica</i> Decne et Planch.	490 m.c.
<i>Evonymus japonicus</i> Thunb.	430
<i>Adiantum japonicum</i> Blume	430
<i>Skimmia japonica</i> Thunb.	420
<i>Aucuba japonica</i> Thunb.	390
Mean value of light intensity	432

As expected, the light value of all these plants is very low and is always under 500 m.c.. The compensation point of *Aucuba japonica* is the lowest of all the plants studied here and it does not reach such a low value as 400 m.c.. The value of the shade-plants is less than a half of the foregoing three groups.

Although in these experiments the difference of the light value of the sun-plants and the shade plants was obtained, the light value of few sun-plants, such as *Ginkgo biloba* and *Magnolia praecocissima*, is low, lying in general between the normal sun-plants and the shade-plants. Therefore, the plants with such intermediate values should be considered, as the shade type of the sun-plant in regard to the compensation point.

SUMMARY

1. The compensation point with leaves of woody plants in the constant temperature at 25°C. was measured.

2. The light value of compensation point of summer-green, needle and broad-leaved ever-green plants is in general over 1000 meter candles. Among them the light value of *Rhus javanica* is the highest and it surely reaches to 2000 meter candles. *Cornus Kousa* shows the next highest value of compensation point which exceeds 1700 meter candles and *Thuja orientalis* and *Ilex crenata* var. *typica* f. *genuina* also belong to the plants which require quite an intense light for the minimal assimilation.

3. *Phellodendron amurense*, *Clethra barbinerbis*, *Cornus controversa* (summer-green plants) and *Cryptomeria japonica* (needle plant) belong to the plants which endure weak light intensity and their compensation point does not reach to 1000 meter candles, yet it is always beyond 800 meter candles.

4. Although *Ginkgo biloba* and *Magnolia praecocissima* belong to the sun-plant, they tolerate a remarkably low light intensity and indeed the compensation point is nearly 700 meter candles.

5. The light value of all the shade-plants experimented here is under 500 meter candles and that of *Aucuba japonica* is the lowest and it reaches nearly to 400 meter candles.

The writer wishes to acknowledge his indebtedness to Prof. Dr. Y. YOSHII, under whose direction this work was done.

LITERATURE

- BOYSEN JENSEN, P. 1918. Studies on the Production of Matter in Light and Shadow Plants. Bot. Tidskr., Vol. 36. (Cited from W. STILE, 1925, Photosynthesis, p. 97).
- 1932. Die Stoffproduktion der Pflanzen.
- und MÜLLER, D. 1928. Über neue Apparate zur Messung der Kohlensäureassimilation, der Respiration, der Öffnungsweite der Spaltöffnungen und der Beleuchtungsstärke. Planta, Vol. 6, pp. 456–472.
- BURNS, G. P. 1923. Minimum light requirements referred to a definite standard. Vt. Agr. Exp. Sta. (Burlington, Vt.), Bull., Vol. 235. (Cited from J. W. TOUMEY, 1928, Foundations of Silviculture upon an ecological Basis, p. 37)
- ERKE, G. 1931. Über die Wirkung der Temperatur und des Lichtes auf der Atmung und Assimilation einiger Meeres und Süßwasseralgen. Planta, Vol. 13, pp. 221–310.
- JOHANSSON, N. 1923. Zur Kenntnis der Kohlensäureassimilation einiger Farne. Svensk. Bot. Tidskr., Vol. 17, pp. 215–223.
- LUNDEGÅRDH, H. 1921. Ecological Studies on the Assimilation of Certain Forest-plants and Shore-plants. Svensk. Bot. Tidskr., Vol. 15, pp. 46–95
- 1924. Der Kreislauf der Kohlensäure in der Natur.
- PLAETZER, H. 1917. Untersuchungen über die Assimilation und Atmung von Wasserpflanzen. Verhandl. phys. med. Ges. Würzburg, Vol. 45, pp. 31–102.
- STÄLFELT, M. G. 1921. Zur Kenntnis der Kohlehydratproduktion von Sonnen- und Schattenblättern. Meddel. fr. Statens Skogsforsöksanstalt, Vol. 18. (Cited from H. LUNDEGÅRDH, 1924, p. 89)

EXPERIMENTAL NOTE ON THE PRESENCE OF ELECTRICALLY EXCITABLE AREAS IN THE REPTILIAN CEREBRAL HEMISPHERE: *CLEMMYS JAPONICA*

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(With four figures)

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I

For many comparative neurologists the cerebral cortex of the reptilian brain has been one of the chief interests because one might expect from the phylogenetical point of view that the hemisphere in this form would give a clue for study to the evolution of the mammalian motor cortex. Most of the writers agree to the fact that in reptiles it is found possible to differentiate the general cortex, of which one can be regarded to be a precursor of the mammalian neopallium. They, however, seem to be of the opinion that it is a matter of much difficulty to be certain as to whether it reveals to any appreciable degree the sign of cortical motor localization. Although so much work has been done from the morphological side, unfortunately we can find but a few among them dealing with experimental method, either of ablation or of cortical stimulation, while even by means of which any general consensus of opinion has not been brought about.

As early as 1916 JOHNSTON¹⁾ accomplished a series of experiments on the cerebral hemisphere of the turtle and the lizard by means of electrical stimulation, yielding the very interesting results that the dorsal pallium corresponds to the motor cortical area in the mammalian brain. He furthermore mentions that in the turtle the "dorsal surface of the olfactory bulb, retraction of the neck, extension of the legs, movements of eyeball and eyelid; dorsal surface of pallium near olfactory peduncle and lateral border of pallium in the anterior one-half or two-thirds of hemisphere, movements of eyes, jaw, neck, legs and tail; striatal area, movements of all parts." (p. 477). He also mentions that no response was obtained from

¹⁾ JOHNSTON, J. B. 1916. *J. Comp. Neur.*, vol. 26, pp. 475-479.

other parts of the surface of the cerebral hemisphere (p. 477). This account, however, is not generally accepted, especially in the hands of KOPPÁNYI and PEARCY.¹⁾ Their chief objection rendered for JOHNSTON's belief is that he carried out the experiment accomplished under deep narcosis and used the one-point electrode. Judging from their own observation, they interpret the JOHNSTON's result of motor response as not of cortical origin but a sub-cortical one. Besides, IVY²⁾ and probably POPA and POPA³⁾ are all of the opinion that the stimulation of the cerebral cortex would not produce motor effect as is the case in birds. On the other hand, the result of BAGLEY and RICHTER⁴⁾ dealing with the alligator seems to support that of JOHNSTON, although awaiting a final decision of whether the motor response observed is of cortical origin or of some others.

Since we thought it worth while to repeat the cortical stimulation experiment such as done by JOHNSTON and others, we carried out a series of experiments dealing with the turtle brain. We were successful in obtaining some electrically excitable areas which are different from those described by the previous writers. The results of the present experiments will be described as follows.

II⁵⁾

The degree of anesthesia renders a point of difficulty in order that no confusion might arise to the reactions following cortical stimulation, since the turtle is hardly narcotized when treated in the usual way. We learned that the best method to follow was first to furnish the animal with hypodermic injection of a dose of urethan (1 cc. per 50 g in 25% solution), and then, cotton wet with ether, if necessary, is applied on the mouth in order to bring the animal into a condition of complete anesthesia. In others, we also tried the stimulation experiment with the animals which had been anesthetized as slightly as possible, just needed for operation with ease. This would not meet the technical objection pointed out by KOPPÁNYI and PEARCY (p. 339). As large a portion as possible of the cranium over the dorsal surface of both hemispheres was removed. When removing the pia mater care was taken so as to minimize the bleeding. When such be the case, cortical stimulation was begun after the bleeding

¹ KOPPÁNYI, T. and PEARCY, J. E. 1924-'25. *Amer. J. Physiol.*, vol. 71, pp. 339-343.

² IVY, A. C. 1929-'20 *J. Comp. Neur.*, vol. 31, pp. 1-13.

³⁾ POPA, GR. T. and POPA, F. GR. 1933. *Proc. Roy. Soc., Ser. B.* vol. 113, pp. 191-195.

⁴⁾ BAGLEY, C. and RICHTER, C. P. 1924. *Arch. of Neur. & Psychiat.*, vol. 11, pp. 257-263.

⁵⁾ To Mr. Y. KITAO our thanks are due for assistance in the experiment.

has stopped.

Cortical stimulation has been accomplished by applying a very weak induction current by either one-point electrode or two-point electrode leading from the inductorium. The one-point electrode which we slightly modified from the SHERRINGTON's¹⁾ unipolar electrode was used, the diameter of platine point being 0.3 mm. so that it would be adequate for such a small brain as that here used. The indifferent electrode consisted of a large sheet of tin plate wet with a saline solution, with which the legs of the animal are in contact.

Prior to stimulation, each area to be stimulated was wiped off with dried cotton in order that the spreading of the current might be prevented as much as possible. Any prolonged stimulation applied on only a limited point must be prohibited, because in such case other reactions not true of cortical origin might come up.

In almost all of the experiments so weak a current as that which could just be detected by the tip of the tongue was used. No stimuli stronger than this was needed for the experiment.

III

In figure 1 are shown electrically excitable areas, stimulation of which gives particular reactions, although the walls of the hemisphere which are situated far caudally and laterally have not been stimulated. Stimulation of area A elicits due movement of a group of neck muscles which serve for the retraction of the neck and head only. The movement as such is on the same side as the stimulation but occasionally it becomes bilateral or involves both sides. All of the turtles experimented on are likely to give this reaction. However, the area under consideration probably is more extensive than that illustrated in figure 1. Figure 2 indicates the arrangement of cortical cells of area A.

Stimulation of area B evokes, though not particularly, the movement of lifting the head. In some cases, by stimulation of the area are caused both the movements of lifting and of retraction of the head, which are probably due to the stimulation applied on the overlapped area as shown in figure 1 or the localization for both movements mentioned above is not well defined.

The most convincing reaction was obtained by stimulation of area C. The stimulation of which is to cause discrete movement of opening of the

¹⁾ FRÖHLICH, J. and SHERRINGTON, C. S. 1902 J. Physiol., vol 28, pp 14-19

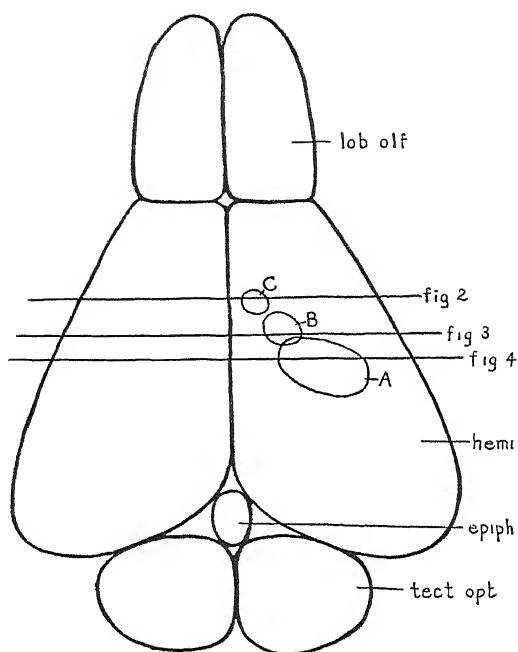


Fig 1. Diagram showing areas A, B and C, stimulation of which causes motor responses. Area A and B, movement of the head and neck, area C, movement of the jaw (opening of the mouth). Area C is magnified approximately in the same proportion as that of the cerebral hemisphere, but areas A and B are magnified roughly. Parallel lines through the cerebral hemispheres indicate the approximate levels of the transverse sections (figs 2, 3 and 4). *epiph*, epiphysis, *hemi*, cerebral hemisphere, *lob. olf.*, olfactory lobe, *tect opt.*, optic tectum. \times ca. 8.

mouth. This was ascertained by applying the one-point electrode carefully in succession to every millimeter from point to point of the dorsal surface of the hemisphere. Even when the current be greatly increased, and when applied upon the rest of the cortex, it does not elicit any characteristic movement of this kind. The area which particularly concerns the movement of the mouth, and which has been measured as above under an assumption that the spread of electrical current might be negligible, is much less than 1 mm. in diameter (figs. 1 and 2). This reaction, however, could not be obtained from all the turtles experimented upon, but two of them gave a very definite response all the time and one gave a possible response. In these animals this response was gained by means of stimulation of the corresponding area but not from other portions of the cerebral



Fig. 2 Transverse section through area C which is indicated in fig 1 Toluidin blue method $\times 16$



Fig 3 Transverse section through area B which is indicated in fig 1. Toluidin blue method $\times 16$

hemisphere, that is to say, area C only in figure 1.

IV

When a comparison is made between figure 1 of JOHNSTON's and that of ours, both of which illustrate the electrically excitable areas, it may be readily seen that there exists rather considerable difference in localization. According to JOHNSTON's figure the excitable areas locate anteriorly of the hemisphere near the olfactory bulb, extending laterally. On the other hand, our result indicates that the area begins at the anterior two-thirds of the hemisphere and extends slightly laterad. Although, for the purpose of localizing the areas following JOHNSTON's figure with great precision, we have tried to stimulate those areas, but it was found impossible to obtain any sign of reactions.

We, furthermore, could not confirm the statement of JOHNSTON that the eyeball, legs and tail have cortical representation. So far as our result indicates, however, we agree with him that the head, neck and jaw have the motor effects following stimulation of the hemisphere.



Fig. 4. Transverse section through area A which is indicated in fig. 1. Toluidin blue method. $\times 16$.

According to our observation and anatomical examination, area A together with area B in figures 1, 3 and 4, both of which concern the mechanism of muscles for movements of the head and neck, may be considered as the most developed without individual difference. Area C (figs. 1 and 2) which is in connection with movement of opening of the mouth may be regarded as a degree of development which varies with individuals, so that the experimental data shows that some animals react but the others do not.

Bearing in mind the suggestion given by KOPPÁNYI and PEARCY that by the use of the unipolar electrode the spreading of the electrical current might be increas-

ed, we began our experiment employing both the unipolar and bipolar electrodes, and in both cases we could obtain the regular responses, in so far as our material permits¹⁾, except that we failed in eliciting the movement of mouth by means of the bipolar electrode. This failure for the latter movement is partly because of impossibility to track out such a small area point to point in succession when applied with the bipolar electrode. We think that the dimensions and the position of the indifferent electrode are correlated with the increase or decrease of the spreading of the current as demonstrated by KOPPÁNYI and PEARCY when the indifferent electrode was placed in the head anterior to the excitation electrode the irregular response was considerably lessened (p. 341). In our experiment we used as the indifferent electrode a sheet of tin plate larger than the animal, so that the difficulty encountered may have been overcome. In this procedure, in reality, the animal showed the regular response.

¹⁾ In this connection, when the snake was stimulated by the same procedure as the case of the turtle, none of the cortical reactions were evoked.

Since we could not experiment upon the animal which is quite free from narcosis, without which operative procedure is found impossible to be accomplished, at present we are unable, with certainty, to answer the second objection raised by KOPPÁNYI and PEARCY (pp. 339, 342). However, we can not be sure that their conjecture proved perfectly correct, in view of the fact that in some mammals any portion of the corpus striatum is electrically inexcitable¹⁾ (WILSON, p. 390).

We must however avoid the final conclusion that the motor response following cortical stimulation is exactly of cortical origin, in so far as the detailed structure of the cortical cells and the precise anatomical pathway from the cortex in question to the lower centers as yet remain unexplored.

¹⁾ WILSON, S. A. KINNIER 1924 Arch. of Neur. & Psychiat, vol. 11, pp 385-404

ON THE BEHAVIOUR OF CATFISH IN RESPONSE TO GALVANIC STIMULI¹⁾

By

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(With two figures)

(Received May 30, 1934)

On keeping a catfish in a small aquarium it will frequently be observed that this fish reacts sharply to stimuli, as slight as the mere touch of a finger against the wall of the aquarium. In the latter case the reaction is such that at the moment of accidental touch of hand or finger against the wall the fish actively jumps in the water as if it were greatly startled. While in other cases the fish is apparently so insensitve that even to a strong knock on the wall of the aquarium it shows no reaction whatever. It was from these facts that HATAI and ABE (1932) suggested the possibility of a relationship between the behaviour of this fish and the occurrence of earthquakes. An observation (HATAI, KOKUBO and ABE, 1932) made somewhat later showed that the catfish has a tendency to become sensitive to a finger knock in association with the rise of potential of the earth current.

Acting on the assumption of a possible relation between the excitability of a fish and the fluctuations of terrestrial galvanic current the present author observed and recorded the reactions of catfish to a controllable galvanic current with a view to ascertaining whether or not the above mentioned sensitivity may be caused by certain laboratorial galvanic conditions. In the present study, therefore, jumping reaction which is thought to be associated with the earth current was observed in relation to the ordinary galvanic stimulus.

As a fundamental phenomenon of excitation the threshold intensity of the stimulus was first determined after which the induction of the fish's jumping reaction due to a weak direct current was tested.

It is with great pleasure that the writer takes this opportunity of thanking The Saito Gratitude Foundation by whose financial aid the present experiment was accomplished. Thanks are also due to Prof. S. HATAI under whose direction the present work was done.

¹⁾ Contribution from the Marine Biological Station, Asamushi, Aomori-ken. No 114.

METHOD

The vessel in which the fish was kept was a small glass tank measuring about 23 cm. in length, 8 cm. in width, and 12 cm. in depth. The electrodes employed for stimulation were of nonpolarisable type of Ag-AgCl as used in their experiments by NOMURA and ISHIKAWA (1933). As in their case the size of the electrodes was roughly 4×4 cm. These electrodes were placed in opposition to one another at either end of the aquarium so as to keep a distance of about 25 cm. Though these electrodes, and consequently through the water and a fish galvanic currents of varying intensities were passed by discharging a condenser of 1 microfarad.

The resistance of the water was carefully regulated so as to maintain a constant 6000 ohms. The time constant of condenser RC was accordingly 0.60σ . The condenser was charged to different voltages thus changing the intensity of the stimulating current. The regulation of the resistance of the stimulating circuit was effected by varying the distance of electrodes from one another or by adjusting the variable resistance inserted in the circuit. The shunt or serial resistance to minimize the resistance change was not, therefore, used. According to the equation $t = RC \log_e n$ roughly 98% of the charge was discharged within 23σ . (Fig. 1). This means that when the condenser was charged to 10 volt it discharged 9.8 microcoulombs of electricity within 23σ . Theoretically the discharge over

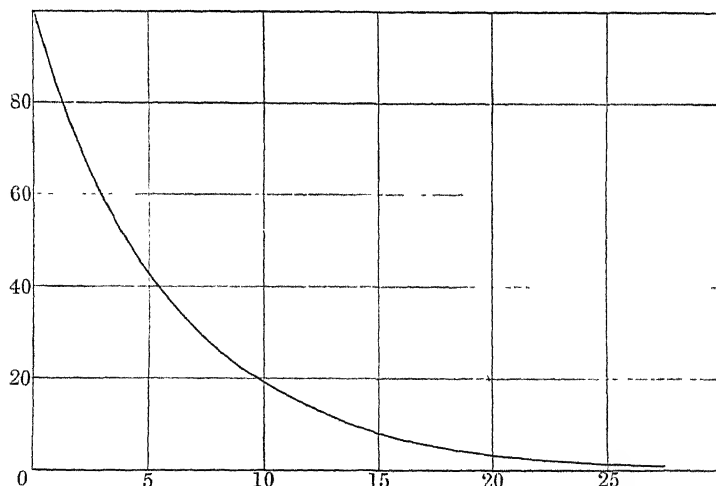


Fig. 1. Discharging curve of a condenser of 1 microfarad through the resistance of 6000 ohms. (ordinate-charge in %, Abscissa-time in σ).

98% shows a considerable delay. So that the final part of the discharge may not have been so effective in stimulating the fish. In the subsequent description, however, voltage of charge i. e. the total quantity of electricity (in micro coulombs) was assumed to be of a stimulant electric quantity.

The stimulation by means of a condenser was made merely for the purpose of determining the threshold potential and the result was expressed in terms of voltage. The voltage reading expressed, at the same time, the electric quantity in microcoulomb, being the capacity of the condenser 1 microfarad.

For the purpose of observing the behaviour of fish to a flowing electricity a weak current of less than 1 milliampere was passed through the water. As a current of this intensity seemed to cause in the electrodes a tendency to polarize a milliampere-meter to indicate net intensity of current was introduced thus enabling actual quantity passed to be measured. When the experiment required the current to be sustained for a long period naked silver electrodes were often employed. This is because the

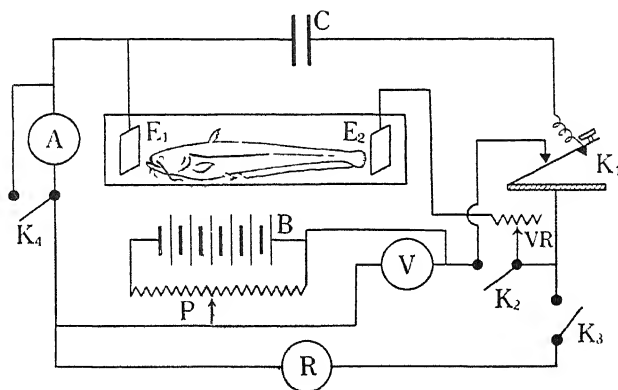


Fig. 2. Diagram of experimental arrangements.

C=condenser, A=mili-amperemeter, E₁, E₂=electrodes,
 B=battery (18 volts), K₁, K₂, K₃, K₄=keys,
 VR=variable resistance (500 Ω), P=potentiometer, V=voltmeter,
 R=resistance meter, Closure of K₁=condenser discharges,
 Release of K₁=condenser charging,
 Closure of K₂=continuous current passes,
 Closure of K₃K₄=(K₁ being closed) for adjusting resistance

long duration of the current only in one direction decomposes the AgCl of the cathode. If so not only does the electrode lose its property of

non-polarisation but Cl is also evolved from the cathode and this mitigates against the success of the experiment if it does not actually kill the fish.

RESULTS

It is observed that when a weak but increasing electric current is passed through the water in which a catfish is kept the fish remains motionless until the current attains a certain intensity. With the increase of current the catfish gradually becomes restless and moves slightly its whiskers and pectoral and caudal fins. The current necessary to provoke the fish to this degree of sensitivity is generally 1 milliampere or less. A further increase of current causes the fish to swim about slowly.

Such is the behaviour of this fish when subjected to a weak electric current. A sudden application of a voltage of, say, 2 or 3 volts stimulates the fish to such an extent as to throw it into convulsions or cause it to jump in the water. As is often the case with other animals the reaction of catfish differs considerably in relation to the direction of current. According to NOMURA and ISHIKAWA (1933) the threshold potential of the catfish for producing minimal response is 2.1 volts when the cathode is placed anteriorly (ascending current), and 2.61 volts when the cathode is placed posteriorly (descending current).

Of the responses of this fish to external stimuli jumping movement is one of the most distinct and clear cut. Not only is this so but a condition which would produce a jumping reaction by knocking may also be provoked by natural conditions. In the present study, therefore, the jumping reaction due to electric stimulus was observed with the object of revealing the nature of this behaviour. For this purpose the threshold potential of the stimulus was observed passing both an ascending and a descending current. The results thus obtained have been tabulated as follows. The figures in the following table represent the threshold voltage of the jumping reaction. These figures can also be read as the galvanic quantity (in micro coulomb) inasmuch as the condenser had a capacity of 1 microfarad.

As has been shown in the above table the threshold for a jumping reaction varies with the individual fish. With an ascending current the threshold was 3.8 volts (i. e. 3.8 microcoulombs for a duration of 23σ) as the mean of 20 different fishes. While in the case of a descending current the threshold potential was much higher than with the ascending current, averaging 10.5 volts (10.5 microcoulombs).

TABLE I

No of fishes	Ascending current (volt)	Descending current (volt)	Sideward current (volt)	Current intensity for sensitivity (mili amp.)	Size of fishes	
					Length (cm)	Weight (gms)
1	2.3	6.5	—	—	16.5	67.5
2	4.0	10.0	—	—	21.7	111.0
3	2.5	6.0	—	—	14.5	58.0
4	7.0	15.0	—	—	23.0	125.0
5	2.3	6.0	—	0.30	18.5	92.0
6	2.7	12.0	—	0.30	17.5	85.0
7	3.5	14.0	5.5	0.10	21.7	120.0
8	5.0	12.0	—	0.80	20.7	103.0
9	2.7	11.0	—	0.18	21.2	109.0
10	5.5	13.0	—	0.52	21.0	110.0
11	3.0	9.0	—	0.09	21.4	114.0
12	2.5	5.0	6.0	0.50	21.5	140.0
13	3.7	12.0	5.2	0.10	21.0	92.0
14	5.0	15.0	6.5	0.18	25.0	91.0
15	3.2	12.0	7.0	0.30	23.5	125.0
16	4.0	8.5	—	0.70	20.0	61.0
17	5.3	11.0	5.0	0.40	21.0	60.0
18	4.7	8.0	2.5	0.60	17.5	37.0
19	4.5	13.0	5.0	0.70	20.4	59.0
20	2.7	11.0	—	0.10	19.0	99.0
Mean	3.8	10.5	5.3	0.37	20.3	93.0

Not only does the threshold vary definitely with the direction of the current but the mode of reaction of the fish also differs accordingly. When the current passed from the anterior of the fish it usually showed a slight indistinct reaction at a threshold far lower than that of the jumping reaction. This minimum response was shown at 2.8 volts in a mean of 20 individuals. From this onward the response augmented in proportion to the increased intensity of the stimulus and at the above shown threshold it showed jumping reaction. In the case of an ascending current the minimum response was not so clearly manifested as with a descending current, and the jumping reaction was invoked more or less suddenly at the threshold above stated. The threshold of minimum response was 3.0 volts, being a little higher than in descending. When the current was passed ascending-wise the fish bent its body backward, while it bent the body ventrally when the current was passed descending-wise. Making observation on *Amblystoma*, LOEB and GARREY (1896) found that this animal bent the body ventrally in response to an ascending current and backward in response to a descending current, thus showing a relation opposite to that of the catfish.

In order to see whether the threshold varies with time observations were made on the same fish at different periods. The results obtained

from the observation of the first fish showed that it changed threshold by 0.5 volts in ascending and 2.0 volts in descending current, within a duration of one day. The fifth fish which was observed for a week showed, in the course of a week, changes of 0.8 volts in ascending and 7.0 volts in descending. These results suggest that there is a tendency for the threshold of the same fish to vary to some extent within a relatively short duration, and that the range of change is wider in the case of a descending than in that of an ascending current. While stimulating the catfish it will be found that the fish can be excited and will show the jumping reaction by a voltage a little lower than the threshold, provided that the stimulus be sent two or more times successively. This was seemingly quite similar to the ordinary summation phenomena of spinal reflex. In the present study, therefore, the stimulating voltage was raised after each push of the discharging key, thus avoiding any banking-up of the previous stimuli. It was also of interest to note that after the catfish had once reacted to a threshold it reacted to a lower voltage on successive stimuli. It seemed as though the resistance of the nerve fibre which passes the impulse was lessened by the first reaction.

Regarding the behaviour of the fish in relation to the direction of current it was observed that in response to an ascending stimulus of 7 volts or so the catfish opened its mouth reflexly, at the same time jumping as if snapping at prey. In the case of a descending stimulus somewhat higher than the threshold, say 15 volts or more, the fish, on the contrary, closes its mouth reflexly. This latter reaction was apparently observed when the fish's mouth was still gaping from the effect of a previous strong ascending stimulus. When, however, the intensity of a descending current was abnormally high, say some 100 volts, the gaping reaction again occurred.

Besides experimenting with ascending and descending currents as above the effect of a sideward current was also examined. As the electrodes were not wide enough to cover the whole body of the fish the stimulus was applied to three parts separately i.e. the head, the trunk, and the tail. Regarding these three parts specifically it was found that the threshold was on an average, lowest in the head, highest in the tail and intermediate in the trunk. In the 19th fish tested the threshold was respectively 5, 8 and 11 volts from head to tail. As the reaction of the trunk and tail was often very irregular the determination was suspended. For this reason the threshold of only the head is given in the foregoing table. These figures show that the threshold of a sideward current is

higher than that of an ascending current and lower than that of a descending current. Besides the relation above mentioned a fact worthy of note was that when a current a little below the threshold was employed the head was always attracted to the anode. Whether the current came from the right or from the left no difference in reaction was observable.

Thus far the behaviour of catfish, especially their jumping reaction, in relation to the galvanic stimulus has been observed. In what follows attention has been directed to the jumping reaction provoked by the rap of a finger against the wall of the tank. As was stated in the foregoing page the catfish in its natural condition is frequently in such a state as to show a jumping reaction in response to a finger rap on the wall of its tank. Examining this phenomenon critically it was found that usually the jumping occurs only once for each rapping. In other instances, however, the fish may jump two or three times when the knocks are made at an interval of a few seconds each. Very occasionally it shows a jump for each knock thus continuing several times of reaction provided the knocks were made several times successively. But in no case does the reaction continue indefinitely. The frequency of jumping seems to vary according to the environment of the fish. That the stopping of jumping may not be the result of adaptation is surmised from the fact that in one case it may jump but once while in another case it may jump several times. Therefore it was natural to suppose that this reaction might have been caused by some environmental condition. It was also surmised that the fact that the fish reacts differently at different times may be due to variations in the intensity of the influencing factor.

While observing the catfish in different ways it was occasionally found that the fish becomes very sensitive during the passing of a galvanic current of some intensity, and reacts in a manner similar to its reaction in the natural condition. This seemed to suggest that the sensitive condition of this fish may also be brought about by laboratorial galvanic means. From this point of view the experiment was started.

At first different strengths of current were passed through the water by employing the aforementioned arrangement. It was soon found that a descending current of over 1 miliampere causes the fish to lose its natural state. A few miliamperes of ascending current made the fish wriggle with agony, as it was unable to avoid the adverse condition because of the limited size of the tank.

Finally it was found that a current of less than 1 miliampere caused the catfish to become very sensitive and to react to the stimulus of

knocking. The results thus obtained were shown in the Table I. The figure in the table show that the intensity of the current which cause the fish to become excited differs with individual fish, one fish reacting to a current as low as 0.09 miliamperes another at 0.8 miliamperes. The mean of the current intensity showed this value to be 0.37 miliamperes.

Direction of the current is an important factor controllng the excitation of catfish. Only an ascending current induces sensitivity in catfish. If the current is passed in a descending direction the catfish does not become sensitive regardless of the strength of the current. This is of interest when considered in conjunction with the fact that the catfish shows jumping reaction with an ascending current at a far lower threshold than with a descending current. Against the sudden onset of a stimulating current the fish invariably reacted to the descending current, while sensitivity due to the flowing current was caused only when the electricity flowed from the posterior of the fish.

The phenomenon of jumping is an "excitation" while the sensitivity of the fish is merely an "excitable condition". Hence it follows that for the excitation of the fish the aforementioned threshold stimuli were required, but for producing "excitable condition" only a weak passing current was needed. Thus, in this case, both phenomena differ radically from each other, but bear a resemblance in relation to the direction of the current.

All the fish tested increased in sensitivity due to the weak ascending current, but only one fish (the fourth) out of seventeen tested showed no increase of sensitivity. As can be seen in the table the threshold potential of this fish was the highest of all. From this fact the probability of a constant relation between the amperes required for sensitivity and the threshold potential of stimulus was anticipated. But an examination of seventeen individuals failed to substantiate any such relations.

As before mentioned the jumping reaction under natural conditions can be repeated more than once according to circumstances. In cases also when the sensitivity was induced by electricity the catfish behaved in a like manner in the majority of cases. But in the latter case the jumping reaction invariably ceased as soon as the current was discontinued. Various means were tried, but in vain, to test whether it were possible to leave the sensitivity until after the current was stopped. Even during the passage of the current successive raps stopped the reaction, and a period of less than 1 minute was required for the fish to regain sensitivity. If the knocking were made every twenty seconds or so loss of sensitivity was avoided and it became possible to test whether the fish was maintaining

its sensitivity. The examination thus made showed that in some fish the sensitivity lasted as long as the current was continued.

In an attempt to study this relation three experiments were made selecting three fishes out of twenty. The results obtained are shown below :

TABLE II.

Time Intensity Fishes	Time after starting experiment (m minute, h hour)												
	0 _m	5	10	20	30	1 _h	2	3	4	5	6	7	8
	Galvanic intensity (in miliampere)												
7 th fish	0.1	0.1	0.1	0.1	0.08	0.1	0.7	0.1	0.1	0.06	0.06	0.05	0.02
13 th fish	0.1	0.1	0.09	0.09	0.07	0.08	0.08	0.05	0.02	0.01	0.01	0.01	—
20 th fish	0.1	0.07	0.04	0.05	—	—	—	—	—	—	—	—	—

The above experiments were made in such a way that to minimize the current within the range of the sensitivity of fishes. As will be seen from the above figures each fish became, at last, to maintain the sensitivity at a far lower potential than at the beginning of the experiment. The initial current of 0.1 miliampere ultimately became 0.01–0.02 m. a. i. e. from 10–20% of the original flow. The above data show that a current of 10 microamperes (roughly 0.1 microampere per sq. cm. of cross section of the tank), an intensity comparable to the natural current, is sufficient to keep the fish sensitive.

A factor which also must be born in mind is the potential of the above cases. As already mentioned the resistance of water remained at 6000 ohms throughout the experiments. The potential of current in all experiments was, therefore, maintained the voltage corresponding to this resistance. For instance with 10 microamperes the potential was 60 milivolt, a potential not at all improbable in a natural current.

With a view to ascertaining whether all fishes have the characteristic of sustaining sensitivity, observations were made on fifteen fishes.

The results showed that thirteen of the fifteen fishes had this nature. It was also found that not only was this the case but in such a case the strength of current required to produce sensitivity diminished with time throughout the course of the experiments.

SUMMARY

1) The behaviour of catfish in response to galvanic stimulus has been observed and confirmed.

2) The mode and degree of reaction to the galvanic stimulus markedly differ according to the direction of current; ascending current being far more effective than descending current.

3) The threshold of the jumping reaction of catfish is 3.8 volts with ascending current and 10.5 volts with descending current when, determined by the present method.

4) The catfish becomes sensitive for the stimulus of knocking when a galvanic current of about 0.4 miliamperes is passed in an ascending direction. By control this current intensity can be decreased as weak as 10 microamperes, an intensity comparable with an earth current.

LITERATURES

- 1) HATAI, S. and ABE, N. 1932. The Response of the Catfish, *Parasilurus Asotus*, to Earthquakes. (Proceedings of the Imperial Academy, VIII (1932), No. 8)
- 2) HATAI, S., KOKUBO, S. and ABE, N. 1932 The Earth Currents in Relation to the Responses of Catfish. (Proceedings of the Imperial Academy, VIII (1932), No. 10)
- 3) KOKUBO, S., ABE, N., and UZUKA, K. 1933. Response of Fishes to the Change of Environmental Factors. I Relation of Earth Current and Electrical Stimulus to the Behaviour of Fish. (Annual Report of the Work. The Saito Gratitude Foundation. No. 9, December, 1933 pp. 33-37).
- 4) LOEB, J. and GARREY, W. E. 1896. Zur Theorie des Galvanotropismus. II. Versuche an Wirbeltieren (Arch. Gesam. Physiol. LXV, pp. 41-47), indirectly cited from. Winterstein, H. 1913. Handbuch der vergleichenden Physiologie, 4 Bd p. 491)
- 5) NOMURA, S. and ISHIKAWA, K. 1933 Response of Fishes to the Change of Environmental Factors II. Preliminary Experiments in the Measurement of Chronaxie in Fishes (Annual Report of the Work. The Saito Gratitude Foundation, No. 9, December, 1933 pp. 37-42)

THE DURATION OF LIFE OF EARTHWORMS IN WATER AND IN PURE GASES

By

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(With four figures)

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INTRODUCTION

The present paper deals with the duration of life of the common earthworms in water and in pure gases, and the cause of death in regard to physiological and ecological viewpoint.

DARWIN (1881) noted in his book "The formation of vegetable mould" that sick individuals, which are generally affected by the parasitic larvae of a fly, must be excepted, as they wander about during the day and die on the surface. He also states, what is often observed, that after a heavy rain succeeding dry weather, an astonishing number of dead worms may sometimes be seen lying on the ground. In such case it is said that worms could have been drowned, but it does not seem likely from the facts that they can remain alive in water for months; and if they had been drowned they would have perished in their burrows. He therefore believed that such worms were already sick, and that their deaths were merely hastened by the ground being flooded.

COUMBAULT ('07 and '09) observed that *Helodrilus*, *Brachiodrilus*, *Hero*, *Eisenia*, *Hesperodrilus*, *Octolasmus*, *Lumbricus* and *Stuhlmanina* could remain for months in water.

FRIEND ('21) found large numbers of dead worms on the surface of the ground on successive mornings in the middle of March of 1921. The conditions for such an occurrence appear to be warm days and evenings, showers during the night and early morning and then a cold snap, but not necessarily a frost. They do not seem to be suffering from parasitization; they can endure a lower temperature than those at which they die; it can not be a case of drowning.

LANKESTER ('21) writing on the same subject believed the dead worms in surface puddles by the side of paths to have perished due to the lack

of oxygen in the water.

MERKER ('26) in Giessen has repeated the investigations above mentioned and it was established experimentally that tap-water when filtered through earth loses much of its oxygen content. The worms will evade this oxygen-hunger by either going deeper or by coming to the surface. The ultra-violet rays in daylight are so harmful to them, that even in dull weather they will in 2-3 hours be paralysed to such a degree that they can scarcely crawl. They may then be unable to penetrate the ground again, and may creep into puddles of water, which will protect them for a time on account of its turbidity.

MERKER and BRÄUNIG ('27) investigating the respiratory requirements of the common *Lumbricidae* under ultra-violet rays (mercury-quartz lamp), found that the amount of oxygen consumed during the radiation is considerably decreased; after the radiation (which first stimulates, then paralyses, and ultimately kills the worms), if the injury sustained is not too great, the consumption of oxygen increases again.

According to FOCKE ('30) in Marburg, the interpretations of MERKER and others must be incorrect, since he maintains that the wandering of earthworms from their burrows depends upon the change of the osmotic relation between the body surface and body fluids.

Against this opinion, MERKER ('30) raises again such objections that the explanation shown from the experimental standpoint is certainly correct.

In connection with the causes of death, the phenomena of the anabiosis also will be considered. Experiments on the anabiosis of earthworms have been carried out by SCHMIDT and STCHEPKINA ('17) at first and by SCHMIDT ('18) himself. KORSCHIELT ('25) remarks that similar facts probably occur in natural condition as SCHMIDT's results indicate. In worms kept in earth (for the purpose of transplantation and regeneration) a continued condition of dryness caused a considerable diminution in length and volume; while a reduction of length equal to one-third took place in the hot summer of 1911, even though the worms were kept moist; in both cases recovery occurred if the worms were placed early enough under normal conditions.

Recently KIM, a former member of our Biological Institute, investigated the duration of life of worms in pure gases (carbon dioxide, nitrogen, oxygen, and air without carbon dioxide and oxygen). But his investigation was merely a simple test (private communication).

Since May of 1932 I have made observations on the longevity of the earthworms in water and also the effect of the air elements upon the

duration of life of worms. The work was done at the Biological Institute of the Tôhoku Imperial University, Sendai, Japan. In the following pages I shall describe the results of my study.

Before going further, I should like to express my sincere thanks to Prof. S. HATAI and Assistant Prof. S. NOMURA for their kind advice and valuable suggestions given during the whole course of this work.

MATERIAL AND METHODS

The materials used for this investigation are two species of earthworms which are widely distributed in Japan. *Pheretima communissima* GOTO et HATAI lives together under half-dacayed fallen leaves or burrows into the humus soil. *Eisenia foetida* SAV. was employed for the purpose of experimental comparison with the former species. The worm lives also in the humus soil or in faeces set aside on the meadow. The collection for the study was made during the months from May to November a period when the worms are found in adult forms.

My observation upon these earthworms can be principally divided into the following three parts; (1) the length of time in which the worms can endure living in various kinds of water; (2) the length of time in which the worms can remain alive in pure nitrogen; and, (3) the length of time in which the worms can continue living in pure carbon dioxide and in water which dissolves a greater quantity of carbon dioxide than that of the water used in the first experiment.

The methods or the apparatus employed were changed according to the experiment. The observations on the experiments are as stated in the following.

EXPERIMENT

I. *Earthworms in water*

As the fundamental observation I have tested how long the worms can remain alive in running water without any supply of food. The animals were placed in the bottom of each glass cylinder (16 cm in diameter and 22 cm in depth); and for the sake of good circulation the running water was regulated into the vessel with the aid of siphons for drainage. Through the course of the observation, the content of water in each glass cylinder was about 2 liters. The results are shown in Table I:

TABLE I.

Number of worms (volume)	Light condition	Duration of life	Water temp	Notes
10 <i>Eisena</i> each volume 0, 4-0, 5 cc.	a) exposed to room light	from the 13th June to the 2nd Sept 82 days	11-25°C	June 20th 2 animals dead in a)
	b) covered with black paper	from the 23rd June to the 24th Sept. 94 days		
3 <i>Pheretima</i> each volume 4, 8-5, 5 cc.	a) exposed to room light	from the 5th Sept. to the 30th Sept. 26 days	16-25°C	Sept. 8th one of them dead in a).
	b) covered with black paper	from the 5th Sept to the 15th Oct. 41 days		

The movement of the animals diminished on the smooth bottom of the vessel and they generally lay still. Within months they became smaller until death that was probably due to hunger.

For the control to the above observation, I have treated the worms in 2 liters of the stagnant tap-water using the same glass cylinder. The results are as follows: (Table II.)

TABLE II.

Number of worms	Light condition	Water temperature	Duration of life
10 <i>Eisena</i> 0, 4-0, 5 cc	in gloomy place	17-19°C.	6 days
3 <i>Pheretima</i> 4, 8-5, 5 cc.	in gloomy place	22-23°C.	18 hours

In this case, the death of the worms would have been hastened by the decrease of the oxygen content and by the excretions (carbon dioxide and other organic substances) of them. In regard to this, further investigations were carried out.

As the next investigation, the worms were put into test tubes that were filled with the three kinds of water and then corked. These observations are annexed in Table III:

I have also measured the oxygen consumption of the worms by C. RISCH's method — micro determination of oxygen content in water — of the modification of WINKLER's. (One species of *Pheretima* only has been tested.) The results are shown in Table IV.

From the facts of the quantitative determination of the oxygen consumption of the worms, it should be mentioned that they can not respire all

TABLE III.

Species of worms (each body volume)	Kinds of the water	Initial pH of the water	Final pH of the water	Duration of life in mean value	Water temp.	Volume of test tubes
A) <i>Eisena</i> collected from Hayakawa Meadow. 0, 45-0, 55 cc.	Tap-water	6, 9	5, 7	47 hours	16-17°C.	cc. 29, 5 29, 6 31, 1 32, 2 31, 7 29, 5 30, 7 31, 5 32, 2 32, 7 31, 4
	Distilled w.	6, 2	5, 1	39 hours		
	Humus filtered water	6, 5	5, 2	44 hours		
B) <i>Eisena</i> collected from Aono Meadow. 0, 48-0, 8 cc.	Tap-water	6, 9	5, 5	43 hours	16-17°C.	
	Distilled w.	6, 2	5, 3	23 hours		
	Humus filtered water	6, 5	5, 5	70 hours		
C) <i>Pheretima</i> 3, 4-7, 0 cc	Tap water	6, 9	5, 75	3 hours 43 min.	21-24°C.	
	Distilled w.	5, 7	5, 55	3 hours 20 min.		

Notes Indicator used 6,8-8,4 Phenol red
5,2-6,8 Brom cresol purple
4,4-6,0 Brom cresol green

Notes of A) and B).. About 10 min. after stuffing the worms wriggle violently About 4 hours after plugging the worms still move At about 30 min. before death, some parts of the body swell up with water About 2 hours after death, hemorrhage occurs in every part of the body that has been swelled up

Notes of C).. 3 min after stuffing the worms wriggle violently. In about 1 hour after plugging the worms still move. At about this time the worms project the male pore. It is very distinct. In about 2 hours after death hemorrhage occurs in the body wall at random.

In both cases the worms elongate their body at death

TABLE IV.

Date	Kinds of water	Water temp	Volume of test tubes in cc.	Volume of the worms in cc.	Water volume in net in cc.	Remain- ed oxy- gen in %	Initial oxygen content in the water in %	Oxygen consumed in %
11th Oct.	Tap-water	18°	29, 5	5, 0	24, 5	0, 095	0, 648	0, 577
			29, 6	6, 1	23, 5	0, 047		
	Distilled water	18°	31, 1	5, 0	26, 1	0, 057	0, 572	0, 484
			32, 2	4, 0	28, 2	0, 118		
12th	Tap-water	17°	29, 5	5, 0	24, 5	0, 054	0, 651	0, 542
			29, 6	4, 1	25, 5	0, 163		

Oct	Distilled water	17°	31, 1	5, 4	25, 7	0, 109	0, 650	0, 589
			32, 2	4, 0	28, 2	0, 030		
13th Oct	Tap-water	17°	29, 6	5, 0	24, 6	0, 055	0, 652	0, 603
			29, 5	5, 0	24, 5	0, 042		
	Distilled water	17°	31, 1	4, 0	27, 1	0, 093	0, 620	0, 558
			32, 2	4, 8	27, 4	0, 031		

oxygen in the water.

I have also placed the worms in liquid paraffin. The diffusion of gas occurs in the oil much more slower than in water. When the worms were immersed in the oil, they wriggled about until suffocation has led them to death. One *Eisenia foetida* died in the oil about 5 hours later.

By adding a few drops of brom cresol green to the tap water in which the worms were placed, I have observed also whether the indicator penetrates into the body or not. The penetration of this indicator took place only through the swelled parts of the body wall about half an hour after death.

II. Earthworms in pure nitrogen

The apparatus for this experiment is shown in figure 1.

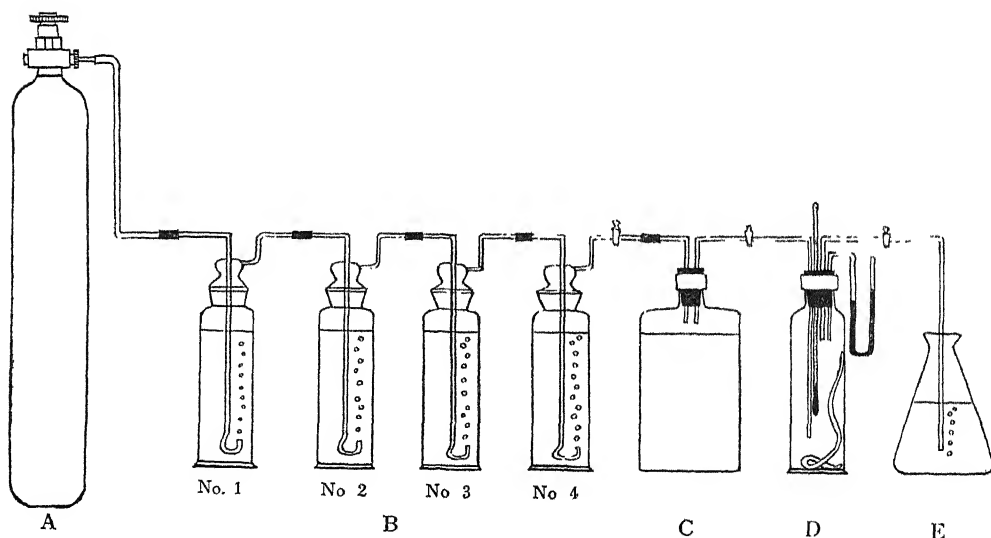


Fig. 1. The apparatus for the experiment of the pure nitrogen. Explanation in the text.

In figure 1., (A) is the bomb tamping with the industrial nitrogen gas. (B) is the washing bottle for the purpose of cleaning the gas. No. 1. of (B) is the solution of potassium permanganate (KMnO_4) by which organic substances are absorbed. No. 2. of (B) is the saturated corrosive sublimate (HgCl_2) with which arsenic compounds can be absorbed. No. 3. of (B) is the pyrogallol solution with caustic potash alkalined, which absorbs oxygen gas. No. 4. of (B) is the caustic potash by which carbon dioxide can be absorbed. (C) is the glass bottle containing the distilled water with which humidity is given to the sending gas to some extent. (D) is the glass cylinder (about 200 cc. content) provided with one thermometer and an open manometer. The animal is tested in (D). (E) is the ERLIENMEYER flask with water from which excessive gas flows out.

At first I placed the earthworms in the glass cylinder (D) and then the bomb and all the bottles were connected with thick gums and slender glass tubes, and all the cocks on the glass tubes were opened. In succession I have opened the stopper of the bomb very carefully, and let the nitrogen run out for 5 minutes. While stopping the cocks that belong to (D), the stopper of the bomb was also closed. Thus I have measured the duration of life of the worms in pure nitrogen gas. Table V shows the results of the experiment.

TABLE V.

Species of the worms (volume)	Temp. in (D)	Reading of manometer	Duration of life	Control in air	Notes
3 <i>Eisema</i> 0, 4-0, 5 cc	21-22	cm 0, 4-0, 5	From the 14th July to the 28th July. 15 days	/	Animals moved in every morning
1 <i>Pheretima</i> 4, 8-6, 0 cc	21-22	0, 5 cm	From the 9th Sept. (P. M. 2) to the 11th Sept (A. M. 10) 44 hours From the 12th Sept. (P. M. 2) to the 14th Sept (A. M. 11) 45 hours From the 14th Sept. (P. M. 1) to the 15th Sept (P. M. 8) 31 hours	54 hours	Animals moved in every morning

III. *Earthworms in the water which dissolves a greater quantity of carbon dioxide than that of the normal condition and in pure carbon dioxide*

Here, I shall research conveniently how long the worms can continue to live in water which dissolves a greater quantity of the carbon dioxide gas than that of the water used in the first experiment. The apparatus for this observation is illustrated in figure 2.

TABLE VI.

Kinds of water	Tap-water				Distilled water				Humus filtered water			
	Initial pH	Final pH	CO ₂ content initial %	Duration of life in min	Initial pH	Final pH	CO ₂ content initial %	Duration of life in min.	Initial pH	Final pH	CO ₂ content initial %	Duration of life in min
With CO ₂ saturated $\frac{1}{2}$ diluted	4,9	4,9	57,39	14	4,2	4,2	23,88	12	5,0	5,0	49,30	15
	5,3	5,3	24,85	50	4,4	4,4	9,97	49	5,4	5,4	29,56	52
3/4 diluted	5,5	5,5	9,99	102	4,7	4,7	5,30	83	5,6	5,6	11,49	118
7/8 diluted	5,6	5,6	5,10	135	5,0	5,0	2,83	115	5,8	5,7	5,73	136
15/16 diluted	5,8	5,7	1,67	160	5,3	5,3	1,70	137	6,0	5,7	1,86	200
normal	6,9	5,75	0,31	223	5,7	5,7	0,19	200	\	\	\	\

Notes. Water temperature. 13-16°C Indicator used. 6,8-8,4 Phenol red. 5,2-6,8 Brom cresol purple 4,4-6,0 Brom cresol green, 4,6-3,0 Brom phenol blue.

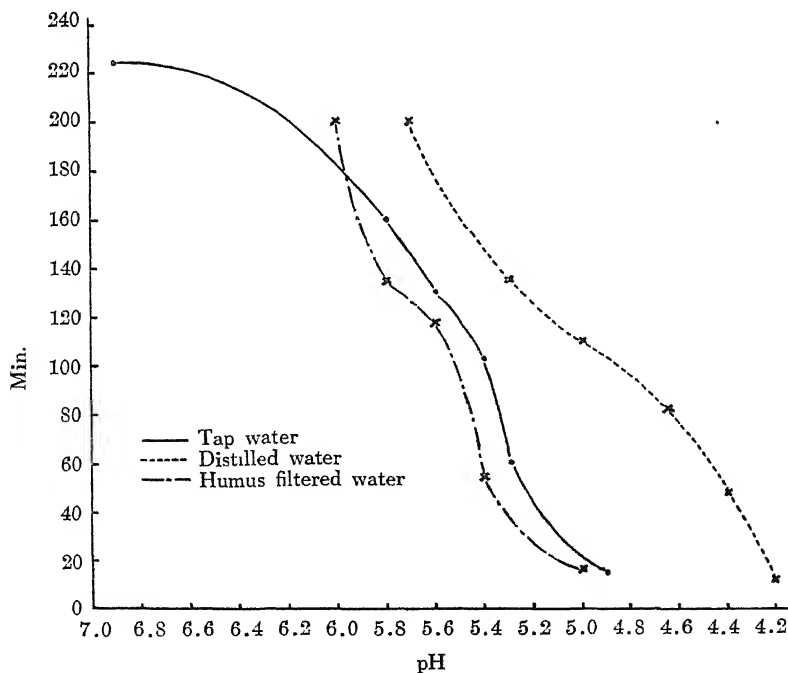


Fig. 3. The relation between the duration of life and hydrogen-ion concentration. (Reference to Table VI)

TABLE VII.

Species of the worms (volume)	Temp. in (D)	Duration of life	Reading of manometer	Notes
1 <i>Pheretima</i> 4, 0-5, 0 cc	13-14°	5-11 min 8 minutes and 30 seconds in mean value	0, 5 0, 6 cm.	Until the short time before the death, the worm was wriggling.

into (D) and the gas left open until the worm died. The results are shown in Table VII.

The worms that were taken out of the current of the carbon dioxide recovered their normal state if they were not exposed to the gas for more than 5 minutes.

Connecting, within 2 minutes, the glass bottle of the capacity of 200 cc. air with the glass cylinder (D) in figure 1, in which the worm and the carbon dioxide were already closed, I have also measured how long the duration of life of the animal will be prolonged. Next, instead of that bottle, I have substituted the bottle of the capacity of 2200 cc. air and repeated the same process. No change of longevity of the worm in either case was recognized.

DISCUSSION AND CONCLUSION

Earthworms can endure submersion in water for long periods. DARWIN quotes from PERRIER, who kept earthworms alive for nearly four months completely submerged. COMBOULT ('09) also kept worms in clear water for months. I have reserved the worms in the running water for months. With the month they became smaller. Death in this case may be probably due to hunger because the supply of oxygen was sufficient. Moreover, from the facts that when placed in 2 liters of stagnant water, 10 *Eisenia* could remain alive about 6 days only, and 3 *Pheretima* about

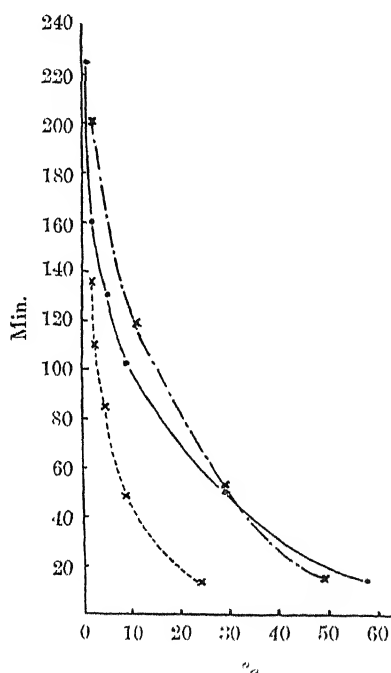


Fig. 4. The relation between the duration of life and carbon dioxide content (Reference to Table VI)

18 hours, we can assume that death was hastened by the decrease of oxygen content in the water and by the effect of the excretions of the animal. (carbon dioxide, and other organic substances.) For the sake of exact experimental proof, I have made observations on the duration of life of animals in test tubes and quantitative determination of their oxygen consumption. We may, therefore, infer from this physiological experiment that the oxygen in the water has been almost exhausted by the worms, and carbon dioxide and other substances have been excreted by the animal, as indicated by the increase in hydrogen-ion concentration (Table IV).

It was proved by MERKER experimentally that tap-water, when filtered through earth, loses much of its oxygen. For this experiment, a glass tube which has a diameter of 5 cm. and a length of 120 cm. was used. 100 cm. of this tube was filled with earth and filter-paper was placed at its bottom. Test was made with tap-water. An example of the results shows the following :

The initial oxygen content in the tap-water	10,004 mg. in L.
Oxygen content in the tap-water filtered through the	
earth in the tube	5,953 mg. in L.
The loss of the oxygen	4,051 mg. in L.
	or 2,331 cc. in L.

Judging from this quantitative determination, MERKER maintains that rain water will lose its oxygen content in a few hours in the soil and the air of the burrows and other crevices of the soil is replaced after a heavy shower by deoxygenated water, and a zone of air-hunger for the inhabitants of the soil will be produced. The worms which can not evade this oxygen-hunger by going deeper will come to the surface of the earth and wander about in daylight. He has also tested the effects of the ultra-violet rays in daylight which has the intensity of 0,0389-0,678 in BUNSEN-ROSCOE's unit and the effects of the artificial light of the mercury-quartz lamp which has the intensity of 0,240-0,432 in the same unit, upon the earthworms. The ultra-violet rays are harmful to the worms, so that even in dull weather they will be paralysed in 2 to 3 hours to such a degree that they can scarcely crawl.

Loose soils contain considerable amounts of water and air in the space between the solid constituents. The soil as the environment of organisms is a complex of many factors which have not completely been analyzed.

The longevity of worms in the water, which contains a quantity of carbon dioxide, or that in pure gaseous carbon dioxide is very short

(Table VI, Figs. 3 and 4). I would state that the earthworms as well as fishes and other aquatic animals are more sensitive to variations in the carbon dioxide content of water than to those in oxygen. Carbon dioxide, if present in quantity, may suffocate animals. Gaseous conditions in the environment may stimulate particular activities and the gases in soil are particularly important for animals that live there. Aeration is necessary, and burrowing animals therefore prefer loose soils.

The results of my experiment, where the worms have been placed in pure nitrogen or in liquid paraffin, have shown that the earthworms can live without any supply of oxygen for considerable periods of time, though the length of survival differs by the genus. KONOPACKI has reported also the same fact by the experiment in which the worms were placed in pure hydrogen gas. In my first experiment, the oxygen content in about 30 cc. of water in the test tube is $0,648 \cdot \frac{30}{100} = 0,1914$ cc. (18°C). And it must be consumed out in less than one hour, if the animal respires constantly 0,2 cc of oxygen per hour, as under normal conditions. But the animal in it could remain alive for about 4 hours. So the animal must have resisted to the unfavorable condition for about 3 hours at least. We must therefore assume that intramolecular respiration is taking place during this period of time, and the same process may perhaps go on ordinarily along with aerobic respiration.

At the last page of my discussion I shall call attention to the phenomena that the male pores in *Pheretima* were protruded very distinctly in the experiment with plugged test tubes, and that disintegration of the body begins to appear at the place just mentioned.

Deriving from my experiment I want to state the conclusion that, even though the cause of the death of earthworms be complex in nature, the lack of oxygen will be the first move to the way of death and the carbon dioxide may affect on the worms as the direct cause of death. Owing to the air hunger and the effect of carbon dioxide, they give up their dwelling place.

SUMMARY

1. Earthworms can endure submersion in running water for months, even though there is difference in their longevity according to the species of the animals.
2. The duration of life in pure nitrogen gas and in pure carbon dioxide gas has been measured.
3. When the animals are placed under unfavorable conditions, they

can maintain life by anaerobic respiration for considerable periods.

4. As the cause of their death, the lack of oxygen will be the first move and the carbon dioxide may give to the worms the direct effect of death.

LITERATURE

- CHAPMAN, R. N. 1931. Animal Ecology New York.
- DARWIN, C. 1881 The formation of vegetable mould London.
- DAUSEND, K 1931. Über die Atmung der Tubificiden. Zeitschr. f. vergl. Physiol, Bd. 14, Heft 3.
- DAVIS, G. and SLATER, W. K. 1928. The anaerobic metabolism of the earthworms (*Lumbricus terrestris*) Biochem Jour, Vol. 22, pp. 338-343.
- FOCKE, F. 1930. Experimente und Beobachtungen über die Biologie des Regenwurms, unter besonderer Berücksichtigung der Frage nach der Raumorientierung Zeitschr. f. wiss. Zool, Bd 136, S. 376-421.
- FRIEND, H 1921. Why do worms die? Nature, Vol 107, p. 172.
- GIESCHEN, A. 1930. Beiträge zur Atmungsphysiologie des Regenwurms Zool. Jahrb, Bd. 48, Heft 1, S 211-168.
- KONOPACKI, M. 1907. Über den Atmungsprozess bei Regenwürmern. Bull. internat Akad. Sc. Cracovie, Philog. Hist Philos. K., p. 488.
- KORSCHOLT, E 1924 Lebensdauer, Altern und Tod. 3 Aufl. Jena.
- KORSCHOLT, E 1925. Über Ruhezustände der Regenwürmer. Zool. Anz., Bd 64, S 53-55.
- LANKESTER, E R 1921. Earthworms drowned in puddles. Nature, Vol. 107, pp 329-330
- MERKER, E 1926 Die Empfindlichkeit feuchthautiger Tiere im Lichte. II. Warum kommen Regenwürmer im Wasserlachen um und warum verlassen sie bei Regen ihre Wohnrohre? Zool. Jahrb, Bd. 42, S 487-555.
- MERKER, E 1930. Treibt Atemnot oder Wassernot den Regenwurm aus der Erde? Zool. Jahrb., Bd 48, S. 667-697.
- NAGANO, T. 1934. Gaseous conditions in the soil of the habitat of the earthworm (*Eisenia foetida* Sav.). In preparation.
- PEARSE, A. S 1926. Animal Ecology New York
- SCHMIDT, P. 1918. Anabiosis of the earthworms J Exp. Zool, Vol 27. pp. 57-72.
- SHELFORD, V. E. and ALLEE W C 1913 The reactions of fishes to gradients of dissolved atmospheric gases. J Exp. Zool, Vol 14, pp. 207-266.
- STEPHENSON, J. 1930. The Oligochaeta. Oxford

ON THE GANGLION CELLS IN THE HEART OF THE PEARL OYSTER: *PINCTADA MARTENSI* DUNKER

By

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(With three figures)

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On the study of the heart physiology, it seems very important to determine the existence of the ganglion cells in the heart of the animals. Reports on the existence of the ganglion cells in the heart of *Lamelli-branchs* are very few. DOGIEL (1877) described that the ganglion cells exist in the auricles and at the auriculo-ventricular junctions of *Anodonta* and *Pecten maximus*. CARLSON (1906) observed the ganglion on the reno-cardial nerve at the base of the auricle of *Platydon*. I have reported that the ganglion cells exist in the heart of the common oyster (1934).

The present research was undertaken with the hope of determining the existence of the ganglion cells in the heart of the pearl oyster. The investigation has been done at the Mitsui Institute of Marine Biology.

MATERIAL AND METHOD

The pearl oyster, *Pinctada martensi* DUNKER, is found in abundance in the Bay of Ohura, near Shimoda. The heart of the pearl oyster is contained in the pericardium which is situated in front of the adductor muscle of the body.

After removing the shell carefully, the heart, the pericardium and the visceral ganglia were fixed in the BOUIN's or FLEMMING's strong solution. The materials were embedded in paraffin and sectioned serially at a thickness of 8~13 μ for the purpose of the investigations. DELAFIELD's haematoxylin with eosin or HEIDENHAIN's iron-haematoxylin were found the most suitable stains for the purpose of examining the nervous ganglion cells. Other fixations and staining methods, sublimate, alcohol, formol, bichromate, or MANN's methylene blue eosin, MALLORY's triple connective staining method, toluidin blue erythrosin or impregnation methods also were tried, but satisfactory results were not obtained. For the vital staining, methylene blue and Rongalit white were used. The method of the vital

staining has been already described in my previous paper ('34).

OBSERVATION

The Pericardium (Fig. 1, Per.) is a thin-walled sac lying between the visceral mass and the adductor muscle. The cavity of the pericardium is more spacious on the right side of the heart than on the left, but the left portion is much elongated and extends for a considerable distance along the anterior margin of the adductor muscle.

The Visceral ganglia (Fig. 1, VG.) lie in a slight depression on the postero-ventral surface of the adductor muscle. The ganglia are covered

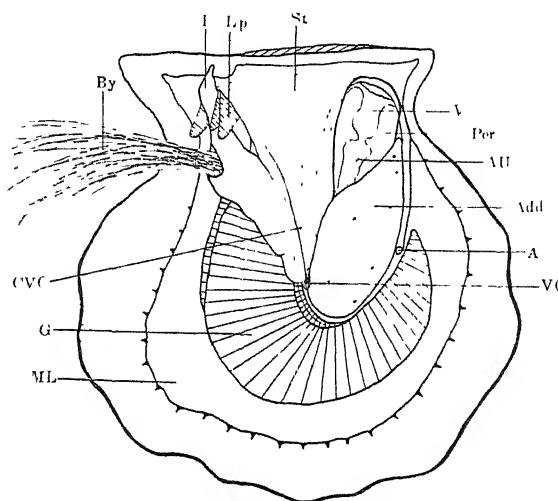


FIG 1 Diagram to show the relative position of the heart, the visceral ganglion, etc. A. anus; Add. adductor muscle; AU. auricle; By. byssus, CVC. cerebro-visceral connectives; F. foot, G. gills, Lp. labial palp; ML. mantle lobe; Per. pericardium, St. stomach; V. ventricles; VG. visceral ganglion.

by a single layer of epithelium. The two ganglia are not fused, namely two separate parts can be distinguished by the naked eye. A stout nerve fibre bundle, visceral commissure, is seen between the two ganglia.

The visceral ganglion consists of a fibrous structure in the centre and nervous ganglion cells in periphery. The fibrous structure of the visceral ganglion is a mass of nerve fibrils and surrounded by many nerve cells. The nerve cell of the visceral ganglion is unipolar and measures about $20\sim 25\mu$ in length and $12\sim 17\mu$ in width. Nucleus of the nerve cell is large and clear in section, almost round in shape and measures about

7μ in diameter, and contains a clear nucleolus. The protoplasm of the nerve cell stains black with HEIDENHAIN's iron-haematoxylin and dark violet with DELAFIELD's haematoxylin, so that they can be distinguished distinctly from the other tissue cells.

The visceral ganglia are connected with the cerebral by means of the cerebro-visceral connectives which lie buried in the connective tissue of the digestive diverticulum, the gonads, and the renal organs. Besides these connectives, the visceral ganglia give off several pairs of nerves innervating the organs surrounding them, the branchial nerves, the adductor nerves, and the pallial nerves. Innervation of these nerves is almost the same as that of the common oyster. The nearest branch to the visceral ganglia of the cerebro-visceral connectives runs towards the pericardium and innervates into the visceral mass after passing the base of the auricles. I have failed to see the penetration of this nerve into the heart even though followed through the serial section.

The Heart of the pearl oyster is, as the common oyster, not traversed by the rectum, and consists of a median ventricle and two symmetrically placed auricles. The heart muscles are better developed in the ventricle than in the auricles. The muscle fibres cross and re-cross each other in all directions, so that the wall of the ventricle has a spongy texture. All the heart muscles are non-striped. The wall of the ventricle is white but the auricle shows very dark brown or black colour. The auricles communicate with the ventricle by a narrow slit on each side which is provided with muscular valves.

The examination of the finer structures of the heart wall had succeeded to reveal the existence of the ganglion cells (Fig. 2). The ganglion cell

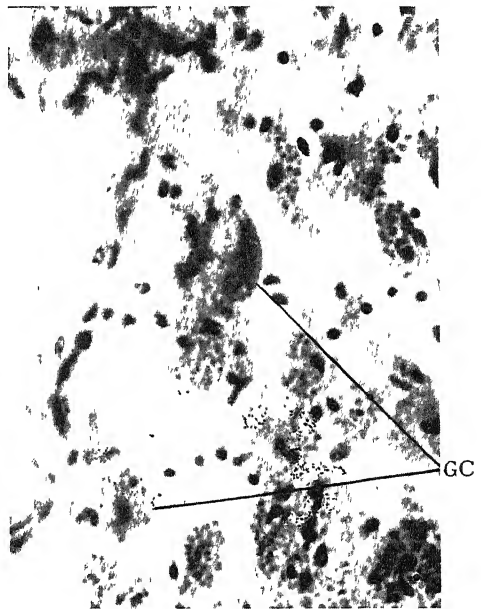


Fig 2 Photograph of the ganglion cells at the auriculo-ventricular junction $\times 650$ GC ganglion cell

in the heart is also unipolar in shape and has a large nucleus which contains a clear nucleolus in its centre. The protoplasm takes a dark violet or black colour with the haematoxylin staining as were noted in the nerve cells of the visceral ganglion.

Fig. 3 illustrates the distribution of these ganglion cells in the heart. That is, in the auricles, there are more ganglion cells in the parts of anterior and posterior, but are in the middle part of the auricles small in number in comparison with the former. At the auriculo-ventricular junction, the ganglion cells arrange themselves in ring-like formation. In the ventricle, the ganglion cells are mainly at the middle part and the anterior, there are no cells in the neighbourhood of the auriculo-ventricular junction.

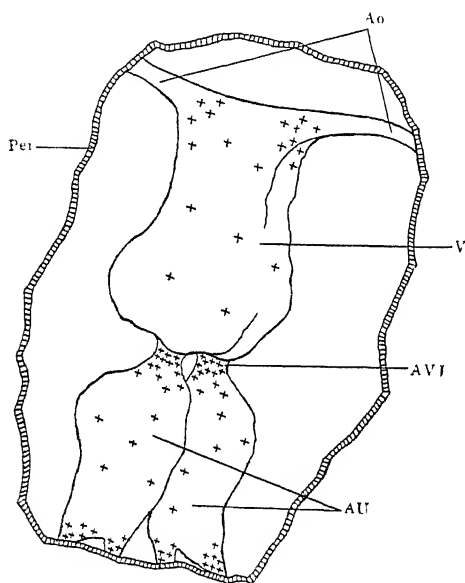


Fig 3 Diagram to show the distribution of the ganglion cells in the heart *Ao* aorta, *AU* auricle, *AVJ* auriculo-ventricular junction, *Per* pericardial wall, *V* ventricle, + indicates localization of the ganglion cells

There are about 60 ganglion cells in the ventricle and about 100 cells in the auricles exclusive of the auriculo-ventricular junction. At the auriculo-ventricular junction, about 100 ganglion cells are discovered. Namely, the number of the ganglion cells is larger in the auricle than in the ventricle.

By using the vital staining with methylene blue and with Rongalit white, I could observe

the nerve fibres in the heart. The fibres in the auricles of the pearl oyster were almost the same in feature as that of the common oyster. In the ventricle, I could not demonstrate the existence of the nerve fibres, for probably the wall of the ventricle is too thick for staining.

SUMMARY

1. The gross anatomy of the pericardium, the visceral ganglia, and the heart is given.

2. The ganglion cells in the heart were discovered and the distribution of these cells were determined.

3. Nerve fibres in the auricles were observed by using the vital staining method. The histological feature of these nerve fibres was almost the same as that of the common oyster.

Before leaving the subject, I desire to express my sincere thanks to Prof. S. HATAI of the Biological Institute of Tōhoku Imperial University for his kind encouragement

LITERATURE

- CARLSON, A. J., 1906 Vergleichende Physiologie der Herznerven und der Herzganglien bei den Wirbellosen. *Ergeb. Physiol.*, Bd. 8.
- DOGIEL, J., 1877. Die Muskeln und Nerven des Herzens bei einigen Mollusken. *Archiv. f. mikros. Anat.*, Bd. 14.
- KRUG, C., 1922. Morphologie und Histologie des Herzens und Pericards von *Anodonta cellensis*. *Zeitschr. f. wiss. Zool.*, Bd. 119.
- SUZUKI, S., 1934 On the Distribution of Ganglion Cells in the Heart of the Oyster. *Sci. Rep. Tōhoku Imp. Univ.* 4th Ser., Vol. 8, No. 4

ON THE INNERVATION OF THE HEART OF LIMPETS

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(With two figures)

(Received June, 12, 1934)

The purpose of the present investigation is to examine the innervation of the heart and to determine the existence of the ganglion cells in the heart of the limpets. The experiments have been done at the Mitsui Institute of Marine Biology.

MATERIAL AND METHOD

The observations upon the heart have chiefly been made from two marine species, *Cellena nigrolineata* (REEVE) and *C. eucosmia* (PILSBERY), which are commonly found in the neighbourhood of the Institute.

The shell was removed carefully and the heart with surrounding tissue was dissected, then fixed by using several fixatives, including: BOUIN's solution, acetic sublimate, formol, ZENKER's solution, and pyridin. Various staining methods were used; DELAFIELD's haematoxylin with eosin, HEIDENHAIN's iron-haematoxylin, MALLORY's triple staining mixture, and toluidin blue with erythrosin. Vital staining with methylene blue and with Rongalit white were also tried but the results were not satisfactory.

DESCRIPTIVE

In this experiment, I used two species of limpets, *Cellena nigrolineata* (REEVE) and *C. eucosmia* (PILSBERY), but the former only will be described, because there was found no difference in the results between them.

When the shell was removed, the heart of the limpet is seen through the pericardial wall. The rate of the heart beat is slow with an average of about 15 to 20 contraction per minute at the room temperature (15°C.).

The blood is a colourless fluid in which float amaeoboid corpuscles. The blood corpuscles are small and colourless, and are about 8μ in diameter and in permanent preparations, show a prominent nucleus in the centre of a rounded or ovoid body.

Pericardium. The pericardium is roughly triangular in shape and completely filled by the heart and situated at the left anterior corner of the dorsal surface of the visceral hump. The walls of the pericardium are muscular and, at the outermost layer, are densely arranged many small but somewhat elongated epithelial cells which stain only with haematoxylin. The ventral wall of the pericardium is thicker and more muscular than that of the dorsal and has numerous eosinophile glands which open to the nuchal chamber. In the ventral wall of the pericardium, a stout nerve fibre which originates from the visceral ganglion runs parallel to the long axis of the heart, namely from the right to the left side of the body.

Visceral ganglion. A visceral ganglion lies to the right of the fore-gut and to the ventral portion of the right posterior corner of the pericardium. It consists of nerve fibres and nerve cells and gives off several nerves. A stout nerve, the visceral nerve, is richly branched and supplies the left kidney, rectum and various viscera. Another stout nerve goes to the right kidney. Other small nerves of the ganglion run into the visceral hump.

The nerve cells of the visceral ganglion are uni- or bipolar in shape. Each nerve cell has a large clear nucleus, and measures $18\sim 25\ \mu$ in length and $7\sim 14\ \mu$ in width. The nucleus measures about $6\ \mu$ in diameter and contains a nucleolus in its centre which stains distinctly dark violet or black with haematoxylin.

Heart. The heart of the limpet consists of a thin-walled auricle in front and a thicker-walled ventricle behind. On the posterior portion of the ventricle, continues an intra-pericardial aortic bulb. All these parts of the heart are white and have no pigments.

The auricle is larger than the ventricle, but its muscular fibres are fewer in number and its walls are thin, transparent and extensible. The auricle gives off a branchial vein from its left anterior corner. This branchial vein soon divides to anterior and posterior branchial veins in front of the pericardium. The anterior border of the auricle communicates with the nuchal chamber through the pericardial wall by several veinlets.

The ventricle stretches right across the pericardium, and its antero-dorsal wall is thicker than that of the postero-ventral. Its dorsal wall is connected by fibres with the roof of the pericardium, along a line going obliquely from right to left. The aperture between the ventricle and the auricle is guarded by two valvular flaps which project into the ventricle cavity. The anterior and posterior aortae start from the right and left corner of the intra-pericardial aortic bulb respectively and they run parallel to and in close connection with the ventricle wall. The walls of the aortic

bulb are thick and muscular almost of the same degree as the ventricular walls. The aperture between the ventricle and the aortic bulb is guarded by a muscular septum which projects into the cavity of the latter. All the heart muscles are non-striped.

Innervation of the Heart. By the studies of serial sections, I could see that the nerve fibre enters the heart through the communicating portion between the auricle and the pericardium. This nerve fibre which innervates the heart is a branch of the visceral nerve. A branch of the visceral nerve runs in the ventral wall of the pericardium and gives off several nerve branches. One of these small nerve branches enter the auricle at about the middle portion of the auricle and runs parallel to the anterior border of the auricle for some distance, then divides into two branches. One of the branches runs along the dorsal wall of the auricle and another along the ventral. Then each of them gives off many fine nerve fibres. Some of these fine nerve fibres go to the ventricle through the aperture and innervate the ventricle. Finer innervation of these fine nerves can not be definitely traced, but in Figure 2 the innervation of some relatively stout nerve fibres is shown diagrammatically. The manners of the endings in the heart muscles of these fine nerve fibres can not be decided. Another nerve branch of the visceral nerve enters the intra-pericardial aortic bulb through the communication between the aortic bulb and the pericardial wall and branches immediately and innervate the walls of aortic bulb and the aorta. The nerve fibres which innervate the aortic bulb tend to the ventricle, but whether these nerve fibres continue with those which enter the ventricle from the auricle can not be determine with certainty.

Ganglion Cells in the Heart. The heart of the limpet is supplied by a nerve from the visceral ganglion, as mentioned above, and yet I could observe several ganglion cells in the heart. The



Fig. 1. Photograph of a ganglion cell in the heart of *Cellena nigrolineata*. $\times 600$

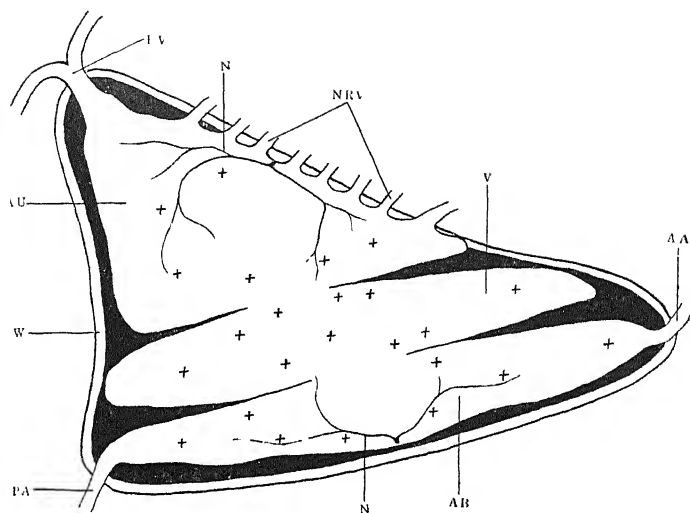


Fig 2 Diagram to show the innervation of the heart and the distribution of the ganglion cells in the heart AA Anterior aorta, AB Aortic bulb, AU, Auricle, N Nerves which innervate the heart, NRV Nemal rootlets; PA Posterior aorta, PV Branchial vein, PW Pericardial wall; V Ventricle + indicate the ganglion cells.

ganglion cells in the heart have been studied also in fixed preparations. They are always easily distinguishable because their size is larger than other tissue cells and their cytoplasm stains darkly with haematoxylin. The nucleus of the ganglion cell is large and contains a clear nucleolus. The ganglion cell is uni- or bipolar and measures $16\sim 22\mu$ in length and $7\sim 14\mu$ in width, and the diameter of its nucleus is about 6μ (Fig. 1).

In the auricle, 4~6 ganglion cells situated in the heart muscles near the auriculo-ventricular junction and 2 cells are always seen on the nerve fibres at the point of entrance of the nerve to the auricle. In the ventricle and the aortic bulb are also discovered several ganglion cells. Some of them are seen on the muscular septum between the ventricle and the auricle or the ventricle and the aortic bulb, and some of them near the aorta. These ganglion cells are always separated from each other by other tissue fibres.

Figure 2 is shown diagrammatically the distribution of the ganglion cells in the heart. There are about 30 ganglion cells in the heart in total. The number of the ganglion cells is larger in the ventricle than in the auricle and in the aortic bulb.

SUMMARY

1. The pericardium is roughly triangular in shape and completely filled by the heart. The ventral wall of the pericardium is thicker and more muscular than that of the dorsal and has numerous eosinophile glands. In the ventral wall of the pericardium, a branch of the visceral nerve runs from right to left of the body.

2. The visceral ganglion consists of nerve fibres and nerve cells. The nerve cells of the ganglion are uni- or bipolar.

3. The heart consists of three parts: the auricle, the ventricle, and the intra-pericardial aortic bulb. The auricle is largest in size but its muscular fibres are fewer than in the other parts of the heart.

4. The nerves which innervate the heart are two branches of the visceral nerve. One enters the auricle and innervates both the auricle and the ventricle, and another enters the aortic bulb and innervates the aortic bulb, aorta, and the ventricle.

5. The ganglion cells in the heart were discovered and the distribution of these cells were determined. The number of the ganglion cells is larger in the ventricle than elsewhere.

LITERATURE

- AINSWORTH DAVIS, J. R. and FLEURE, H. J., 1903 *Patella* (The Common Limpet) L. M. B. C. Memoirs X, London
- CARLSON, A. J., 1906 Vergleichende Physiologie der Herznerven und der Herzganglien bei der Wirbellosen Ergeb. Physiol., Bd. 8.
- DOGIEL, J., 1877 Die Muskeln und Nerven des Herzens bei einigen Mollusken Archiv. f. mikrosk. Anat., Bd. 14.
- KRUG, C., 1922. Morphologie und Histologie des Herzens und Pericards von *Anodonta cellensis* Zeitschr. f. wiss. Zool., Bd. 119
- SUZUKI, S., 1934 On the Distribution of Ganglion Cells in the Heart of the Oyster. Sci. Rep. Tôhoku Imp. Univ. 4th Series, Vol. 8, No. 4.

ÜBER DIE EXOGASTRULABILDUNG BEIM SEEIGELKEIM DURCH AUXIN, GLYKOGEN UND KClO_3

VON

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(Mit 4 Figuren)

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EINLEITUNG

Neue Untersuchungsergebnisse über die Natur des Organisators im Amphibienkeim zeigen, dass die Vorgänge der embryonalen Induktion chemische Prozesse sind.¹⁾ Beim Seeigelkeim wurde von HÖRSTADIUS ('31) festgestellt, dass die Mikromeren starkes Induktionsvermögen besitzen. Wenn die Exogastrulabildung beim Seeigelkeim, wie von HERBST ('92 und '96), v. UBISCH ('29), MACARTHUR ('24) und HÖRSTADIUS ('31) berichtet wurde, ein Merkmal der Vegetativisierung oder der Veränderung der Verhältnisse zwischen dem animalen und dem vegetativen Gefälle des Keims ist, so muss es ein Weg zur Lösung der Frage nach der Wirkungsweise des Induktors sein, die die Exogastrula induzierenden Stoffe in mehreren Zahlen zu finden und ferner deren Wirkung untereinander zu vergleichen.

Die Pflanzenphysiologen²⁾ fanden einen interessanten organischen Stoff Auxin, der das Wachstum der Pflanzen bewirken soll, und stellten fest, dass sich dieser Stoff auch im tierischen Gewebe befindet.

Die Frage ist nun hier, in welcher Weise Auxin auf die tierischen embryonalen Zellen wirkt, und weiter, ob das Glykogen wie beim Amphibienorganisator auch beim morphogenetischen Verlauf des Seeigelkeims irgend eine Rolle spielt. Im Folgenden werden die Ergebnisse meiner Versuche über die Wirkung des Auxins und des Glykogens am Seeigelkeim beschrieben. Dabei wurde ferner auch die Wirkung von KClO_3 geprüft, das ein Oxydationsmittel ist.

Als Material wurden die Eier eines Seeigels, *Strongylocentrotus pulcherrimus* (A. AGASSIZ), benutzt. Der Keim wurde in HERBSTSchem

¹⁾ HOLTFRETER ('33); SPEMANN, FISCHER und WEHMEIER ('33); FISCHER und WEHMEIER ('33); WADDINGTON, J. und D. M. NEEDHAM ('33 und '34); WOERDEMAN ('33a, '33b, '33c und '33d); RAVEN ('33).

²⁾ WENT ('28); KÖGL ('33a und b); BOYSEN-JENSEN ('31); MASCHMANN und LAIBACH ('32).

künstlichem Meerwasser¹⁾ gezüchtet.

EXPERIMENTELLER TEIL

1) *Auxin*. Zur Gewinnung des Auxins wurden die Kulturflüssigkeit von *Aspergillus niger* und menschlicher Harn als Ausgangsmaterial benutzt. Nach der Methode von BOYSEN-JENSEN²⁾ züchtete ich *Aspergillus niger* etwa sechs bis zwölf Tage lang bei 30–35°C. 500 ccm Kulturflüssigkeit wurde nach Ansäuern mit Essigsäure mit Äther³⁾ ausgeschüttelt. Der Ätherextrakt wurde mit gesättigter wässriger Lösung von NaHCO₃ extrahiert. Der Bikarbonatextrakt wurde noch einmal mit Äther ausgeschüttelt. Der letzte Ätherextrakt wurde eingedampft. Der Rückstand dieses Extrakts wurde mit 4,6 ccm destilliertem Wasser gelöst. Diese Stammlösung, welche pH 4,6 ist, wurde in kleine Ampullen getan.

Frischer menschlicher Harn wurde mit Salzsäure sauer gemacht und in der Luft eingedampft. 1200 ccm Harn hinterlassen 200 ccm einer braunen Konzentration. Sie wurde mit Essigsäure angesäuert und mit Äther extrahiert. Weiter wird sie, wie oben beschrieben, behandelt. Der Rückstand des Ätherextrakts wurde mit 6 ccm destilliertem Wasser gelöst und in Glasampullen aufbewahrt.

Die Konzentration von Auxin wurde nach WENTSche Methode (WENT '28) gemessen und zwar in der photographischen Dunkelkammer bei rotem Licht. Die Stammlösung von Auxin wird mit dem gleichen Volumen einer 0,3 prozentigen Agarlösung gemischt und koaguliert. Ein kleiner Teil des koagulierten Auxin-Agar-Gemisches wurde exzentrisch auf die dekapitierte Koleoptile von *Avena sativa*, welche vorher im Dunkeln drei Tage lang bei 31°C etiolmiert worden war, gebracht. Kurz danach liess ich das Schattenbild der *Avena*-Koleoptile durch das rote Licht auf die photogra-

¹⁾ NaCl	30,0 g.
KCl	0,8 g.
MgSO ₄ (7 H ₂ O)	13,6 g.
CaCl ₂ (2.H ₂ O)	1,7 g.
NaHCO ₃	0,5 g.
destilliertes Wasser	1140,0 ccm.

²⁾ Ich benutzte die folgende Kulturflüssigkeit nach BOYSEN-JENSEN ('32) für *Aspergillus niger*, ohne Korkpatrikel hinzu zu fügen,

destilliertes Wasser	1000,0 ccm
Traubenzucker	25 g.
Pepton	5 g.
Zitronensäure	0,25 g.

³⁾ KÖGL empfiehlt Benutzung peroxydfreien Äthers. Bei meinem Versuch war käuflicher Äther, KONISHIS *Ether anhydrous, distilled over sodium*, brauchbar.

phische Platte¹⁾ fallen. Nach zwei Stunden wurde wieder belichtet, ohne die Lage der Pflanzen und der photographischen Platte zu verändern. Die Platte wurde dann entwickelt. Die relative Lage der Doppelbilder der sich krummenden *Avena*-Koleoptile im Verhältnis zur Anfangslage wurde nach der entwickelten Platte gemessen. Durch diese Versuche stellte ich fest, dass meine Stammlosung von Auxin aus *Aspergillus* zehnfach stärker als die aus menschlichem Harn gewonnene war.

Die Eier von *Strongylocentrotus pulcherrimus* wurden zehn Minuten nach der Befruchtung in die Versuchslösung gebracht und weiter gezüchtet. Bei einem Versuch wurde 1 ccm Stammlosung von Auxin aus *Aspergillus* mit 20 ccm HERBST'schem Meerwasser verdünnt. Bei einem anderen Versuch wurde die Stammlosung von Harn-Auxin wie oben etwa 20 f. ch. verdünnt. Bei allen Fällen wurden die Keime in diesen Lösungen bis zum Ende der Versuche gezüchtet und mit dem Kontrollkeim oder mit dem Lithiumkeim verglichen.

Die Furchung war in diesen Lösungen normal. Zwei Tage nach der Befruchtung war die Kontrollkeim im Gastrulastadium und nach drei Tagen im Pluteusstadium (Fig. 1). Beim Auxinkeim begann die Gastrulation nach zwei Tagen. Aber er wurde die Exogastrula oder die Exoentogastrula von verschiedenem Grade. Der in der Auxinlösung des menschlichen Harns gezüchtete Keim war wegen der schwachen Konzentration des wirksamen Stoffs meist die Exoentogastrula (Fig. 2). Der entodermale Bereich wird vom Gastrulakörper in der halben Länge des Urdarms abgestossen, und der überbleibende entodermale Bereich invaginiert in normaler Richtung. Nach drei Tagen war der Keim noch im Exoentogastrulastadium geblieben. Einige Schnürungen wurden im entodermalen Bereich beobachtet. Ein zapfenförmiger Auswuchs hatte sich am animalen Pol der Gastrula gebildet. Aber die Arme des Pluteus und das Larvenskelett entwickelten sich nicht.

Der in der Auxinlösung von *Aspergillus* gezüchtete Keim wurde die typische Exogastrula (Fig. 3 und 4). Die Umkehrung des entodermalen Bereichs begann nach zwei Tagen. Nach drei Tagen schnürte sich der völlig umgekehrte entodermale Bereich in vier Teile ab, welche mit den Schnürungen des normalen Entoderms von Pluteus übereinstimmen. Die Schnürung zwischen dem ektodermalen und dem entodermalen Bereiche ist so schmal, dass der umgestülpte entodermale Bereich von diesem Punkt an sehr leicht abweicht. Das mittlere Stück des umgestülpten Urdarms

¹⁾ILFORDS Soft Gradation Panchromatic Plate

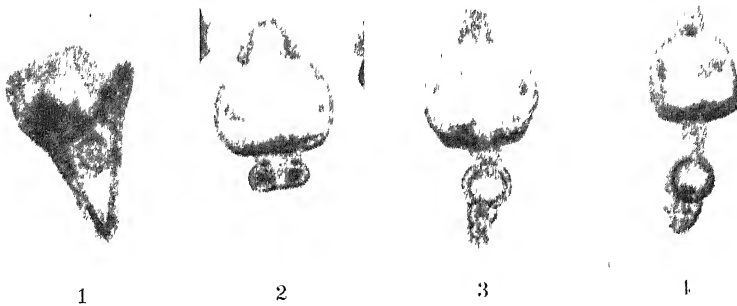


Abb 1-4 Bildung der Exogastrulae bei den Larven von *Strongylocentrotus pulcherrimus* 1 Normaler Pluteus. 2 Exoentogastrula im Harn-Auxin 3 und 4 Exogastrulae im *Aspergillus*-Auxin

ist dickwandig und bildet ein kleine Bläschen, welches vielleicht dem Mitteldarm des normalen Keims entspricht. Der ektodermale Bereich war blasenförmig. Am animalen Pol des dünnwandigen, ektodermalen Bereichs wurde ein zapfenförmiger Auswuchs beobachtet. Der ektodermale Bereich des in der *Aspergillus*-Auxinlösung gezüchteten Keims war klein im Vergleich mit dem in der Harn-Auxinlösung gezüchteten. Auch der zapfenförmige Auswuchs war kleiner je nach der Grösse des ektodermalen Bereichs oder nach der Konzentration der Auxinlösungen.

Einige Verschiedenheiten wurden zwischen dem Auxinkeim und dem Lithiumkeim beobachtet. In dem Gemisch von 1 ccm isotonischer¹⁾ LiCl-Lösung mit 100 ccm HERBSTSchem Meerwasser war keine Wirkung des Lithiums bei *Strongylocentrotus pulcherrimus* zu sehen. Die typische Wirkung von Lithium erschien bei 1,5 bis 5 Volumprozent der LiCl-Lösung. Bei 1,5 bis 2 Prozent wurde der Keim zu einer Exoentogastrula. Erst bei 3,5 Prozent wurde er zu einer Exogastrula. Der Keim bewegte sich aber nicht lebhaft, verglichen mit der Exogastrula der Auxinlösung. Diese schwamm so lebhaft umher, dass der umgestülpte entodermale Bereich oft vom Ektoderm abgelöst wurde. Ferner entwickelte sich beim Lithiumkeim der zapfenförmige Auswuchs am animalen Pol nicht. Mit anderen Worten: die Differenzierung des ektodermalen Bereichs ist beim Lithiumkeim unvollkommen.

2) *Glykogen*. Die neuen Ergebnisse der Untersuchungen über die chemische Natur der Organisatorwirkung beim Amphibienkeim zeigen die

¹⁾ Eine 2,3 prozentige wässrige Lösung von LiCl ist isotonisch mit dem pazifischen Meerwasser in der Gegend von Matusima

Wichtigkeit des Glykogenstoffwechsels. Es ist von SPEMANN und anderen behauptet worden, dass Glykogen mit dem Induktionstoff identisch ist. Im Gegensatz zu ihnen meinen WOERDEMAN ('33a, b, c und d) und RAVEN ('33), dass Glykogen selbst nicht der Induktionstoff sei, sondern dass die glykolytischen Vorgänge im Organisationszentrum für die Bildung des Induktionstoffs eine wichtige Rolle spielen. Jedenfalls muss wohl das Glykogen ein für die morphogenetischen Vorgänge des Keims wichtige Substanz sein.

In meinem Versuch benutzte ich das käufliche Glykogen von SANKYO. Das Glykogen wurde in HERBSTSchem Meerwasser gelöst und der Keim in dieser Lösung gezüchtet. Bei Konzentrationen von mehr als 0,3 Gewichtsprozent Glykogen bildete sich die Exoentogastrula. Bei 1 bis 0,8 Prozent wurde etwas Exogastrula beobachtet. Die morphologische Beschaffenheit dieser Exoentogastrula und Exogastrula ist den Auxinkeimen sehr ähnlich. Der zapfenförmige Auswuchs wurde auch beim Glykogenkeim beobachtet. In konzentrierter Glykogenlösung, die bei meinem Versuche 1 prozentig war, schrumpft der Keim, als ob er plötzlich in hypertonsche Lösung geworfen würde. Aber diese Erscheinung ruht nicht vom osmotischen Druck der Glykogenlösung her, denn solche Erscheinung wird, wie unten berichtet werden wird, auch bei der Züchtung im isotonischen Gemisch von Traubenzucker und HERBSTSchem Meerwasser beobachtet. Kurz, die Wirkung des Glykogens ist schwächer als die des Auxins.

Durch Glykogen wird der Keim in den meisten Fällen zur Exoentogastrula und seltener zur Exogastrula. Aber wenn die Lösung zu stark ist, so schrumpft der Keim, bevor er eine schöne Exogastrula wird. Nun fragt es sich, ob diese eine Exoentogastrula bildende Wirkung des Glykogens von der Unreinheit des benutzten Glykogens verursacht wird. Denn auch wenn Auxin, welches immer in bakteriologischen Nährstoffen, z. B. im WITTE-Pepton, gemischt vorkommt¹⁾, als Unreinheit in meinem Glykogen vorhanden wäre, so wäre doch das Ergebnis meines Versuchs nicht als spezifische Wirkung des Glykogens anzusehen. Also wiederholte ich das Verfahren der Extrahierung des Auxins aus meinem Glykogen, konnte aber keine Spur von Auxin in 5 Gramm Glykogen nachweisen. Ferner war das nach diesem Verfahren wieder mit absolutem Alkohol zurückgezogene Glykogen ebenso wirksam wie das nicht behandelte Glykogen. Deshalb ist die oben beschriebene Wirkung des Glykogens nicht auf die unreine Mischung des Auxins zurückzuführen.

¹ BOYSEN-JENSEN ('31) und BONNER ('32)

Nach Analogie der Muskelphysiologie wurde die Wirkung des Traubenzuckers geprüft. Eine isotonische wässrige Lösung von Traubenzucker wird mit Herbstschem Meerwasser in verschiedenem Verhältnis gemischt und der Keim kurz nach der Befruchtung in dem Zucker-Meerwasser-Gemisch gezüchtet. In einem Fall wurde eine Mischung von Traubenzuckerlösung und HERBSTschem Meerwasser zu gleichen Teilen benutzt. Dieses Gemisch enthält 10 Gewichtsprozent Traubenzucker und ist zehnmal stärker als das Gewichtsprozent der oben beschriebenen Glykogenlösung. Trotz grosser Konzentration des Traubenzuckers entwickelt sich der Keim bis zum schrumpfenden Pluteus. Beim schwächeren Zuckergehalt der Kulturflüssigkeit, welche zwei Gewichtsprozent Traubenzucker enthielt, entwickelte sich der Keim zum guten Pluteus mit normalen Armen und normalem Skelett. Aus diesem Versuch erkennen wir, dass der Traubenzucker keine Exogastrula bildende Wirkung besitzt und dass die Schrumpfung des Keims auf der Mischung von Traubenzucker und HERBSTschem Meerwasser auch beruht.

3) $KClO_3$. Nun wurde die Wirkung von $KClO_3$, das ein Oxydationsmittel ist, geprüft. Ein Gemisch von 5 Volumteilen einer 6,5 prozentigen wässrigen $KClO_3$ -Lösung und 95 Teilen HERBSTschen Meerwassers bewirkt beim Seeigelkeim Bildung der Exogastrula. Eine schwächere Lösung ist wirkungslos und eine stärkere hemmt die Entwicklung. Der Umfang der optimalen Wirkung der Lösung ist bei $KClO_3$ klein. Der durch $KClO_3$ hervorgerufene Exogastrulakeim ist schwächlich. Er bewegt sich schwerfällig auf dem Boden der Glasschale. Die morphologische Beschaffenheit des $KClO_3$ -Keims ist der des Lithiumkeims ähnlich. Beiden fehlt der zapfenformige Auswuchs am animalen Pol. Aber es gelang mir nicht, beim $KClO_3$ -Keim die verschiedenen Stufen der Vegetativisierung zu beobachten, die beim Lithiumkeim eine typische Erscheinung ist.

VERSUCHSERGEBNISSE

In den obigen Versuchen wurde die Wirkung von Auxin, Glykogen und $KClO_3$ geprüft. Diese drei Substanzen bewirken beim Seeigelkeim Bildung der Exogastrula oder der Exoentogastrula. Die Wirkung des Auxins ist spezifisch und stark. Der Keim entwickelt sich zum typischen Exogastrula oder Exoentogastrula je nach der Konzentration des Auxins. Dieser Keim bewegt sich lebhaft im Vergleich zum Lithiumkeime. Mit anderen Worten: die Beweglichkeit der Exogastrula ist beim Auxinkeim am wenigsten geschädigt, im Vergleich zum Glykogen-, $KClO_3$ - und Lithiumkeime.

Eine andere Verschiedenheit ist, dass beim Auxin- und Glykogenkeim deutlich ein zapfenförmiger Auswuchs am animalen Pol gebildet wird. Beim Lithiumkeim von *Strongylocentrotus pulcherrimus* erscheint dieser Auswuchs nicht. Aber bei *Echinus microtuberculatus* und *Asterias glacialis* wurde von HERBST ('92 und '96) die Bildung dieses Auswuchses durch Lithiumsalz beobachtet. Daher können wir es nicht als wesentliche Verschiedenheit der Wirkung des Auxins und des Lithiums ansehen, dass beim Lithiumkeim von *Strongylocentrotus pulcherrimus* dieser Auswuchs nicht gebildet wird.

ZUSAMMENFASSUNG

Die Wirkung von Auxin, Glykogen, Traubenzucker und KClO_3 auf die befruchteten Eier von *Strongylocentrotus pulcherrimus* wurde geprüft. In der Auxinlösung entsteht die Exogastrula. In der Glykogenlösung bildet sich meist die Exoentogastrula. Exogastrulation wird auch durch Zusatz von KClO_3 in HERBSTSchem Meerwasser erzielt. Aber der Traubenzucker besitzt diese Wirkung nicht.

LITERATURVERZEICHNIS

- BONNER, J. 1932 The production of growth substance by *Rhizopus sinuatus* Biol Zbl LII.
BOYSEN-JENSEN, P. 1931. Über Bildung eines Wachstumsregulators durch *Aspergillus niger*. Biochem. Z. CCXXXIX.
FISCHER, F. G und E. WEHMEIER. 1933. Zur Kenntnis der Induktionsmittel in der Embryonalentwicklung. Naturwiss. 1933.
HERBST, C 1892 Experimentelle Untersuchungen über den Einfluß der veränderten chemischen Zusammensetzung des umgebenden Mediums auf die Entwicklung der Thiere I Teil Versuche an Seeigelerien. Z. wiss. Zool LV
— 1893. Dasselbe II. Weiteres über die morphologische Wirkung der Lithiumsalze und ihre theoretische Bedeutung. Mitt. zool. Stat. Neapel XI.
— 1893. Dasselbe. III–VI Theile. Roux' Arch. II.
HÖRSTADIUS, S 1931. Über die Potenzverteilung im Verlauf der Eiachse bei *Paracentrotus lividus* Lk. Archiv. for Zoology. XXIII. B. No 1.
HOLTFRETER, J. 1933 Nachweis der Induktionfähigkeit abgetoteter Keimteile Isolations- und Transplantationsversuche Roux' Arch. CXXVIII
KÖGL, F. 1933. Die Chemie des Auxins und sein Vorkommen im Pflanzen und Tierreich. Naturwiss 1933.
KÖGL, F ; A J HAAGEN-SMIT und H. ERXLEBEN 1933. Über ein Phytohormon der Zellstreckung Reindarstellung des Auxins aus menschlichem Harn. IV. Mitt über pflanzliche Wachstumsstoffe Hoppe-Seylers Z. CCXIV.
MACARTHUR, J W. 1924. An experimental study and a physiological interpretation of exogastrulation and related modification in echinoderm embryos. Biol Bull XLVI.
MASCHMANN, E. und F. LAIBACH. 1932. Über Wuchsstoffe. Biochem. Z. CCLV.
RAVEN, C. P 1933 Experimentelle Untersuchungen über den Glykogenstoffwechsel des

Organisationszentriums in der Amphibiengastrula. I. Kon Akad. Wetens. Amsterdam XXXVI.

- SCHLEIP, W. 1929 Die Determination der Primitiventwicklung. Leipzig
- SPIMANN, H., F. G. FISCHER und E. WEHMEIER 1933 Fortgesetzte Versuche zur Analyse der Induktionsmittel in der Embryonalentwicklung. Naturwiss. 1933.
- UBISCH, L. v. 1929. Über die Determination der Larvenorgane und der Imaginalanlage bei Seeigeln Roux'Arch. CXVII
- WADDINGTON, C. H.; J. NEEDHAM and D. M. NEEDHAM 1933 Physico-chemical experiments on the amphibian organizer. Nature (London) 1933, II.
- WADDINGTON, C. H., J. NEEDHAM and D. M. NEEDHAM 1934 Physico-chemical experiments on the amphibian organizer. Proc. Roy. Soc. London B. CXIV No. B789
- WENT, F. W. 1928. Wuchsstoff und Wachstum. Rec Trav. bot. Neerlandais, XXV.
- WOERDEMAN, M. W. 1933, a. Über den Glykogenstoffwechsel des Organisationszentriums in der Amphibiengastrula. Kon Akad. Wetens. Amsterdam. XXXVI No. 2
- 1933, b. Über den Glykogenstoffwechsel tierischer "Organisatoren". *ibid.* XXXVI No. 4.
- 1933, c. Embryonale Induktion durch Geschwulstgewebe *ibid.* XXXVI No. 5.
- 1933, d. Über die chemischen Prozesse bei der embryonalen Induktion *ibid.* XXXVI. No. 9.

ON THE LOCAL VARIATION IN THE SHELLS OF *MERETRIX* *MERETRIX* (L.), WITH SPECIAL REFERENCE TO GROWTH OF ORGANISM

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(With four figures in text)

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Up to the present time the problems of the relative growth in living organisms have been the subject of investigation by many investigators. HUXLEY has in animals studied quantitatively the heterogonic growth of parts of the body and arrived at important conclusions with regard to growth gradients. In his formula, $y = bx^k$, k expresses a constant differential growth ratio or growth-coefficient of the organ to the growth of the body considered as a standard. KLEIN and SCAMMON (1930) have applied the same formula to the growth of the human body, and GREEN and FEKETE (1933) to the growth of the mouse. Furthermore, a similar formula has been adopted by HERSH (1931) to express the genetic growth of *Drosophila*; and by ANDERSON (1932, '33) for the growth of the body, and for the rate of regeneration of wounds in the carapace, of *Daphnia magna*.

In general, the relation between length (L) and mass (W) can be represented by $W/L^3 = K$, where K denotes a constant. PÜTTER has applied this relation to the growth of the plaice, [*vide* THOMPSON (1917), PRZIBRAM (1922), and JANISCH (1927)], and THOMPSON has discussed the growth of the plaice in his book with regard to the changes of the constant K . CROZIER (1914) has observed that the linear relation $y = ax + b$ exists in the shells of *Dosinia discus* (REEVE), where x denotes length and y depth, width, etc.; and he has proved that $(W + D)/L = K$, where K denotes a constant, W width, D depth, and L length. Such relations as have just been mentioned may obviously be generalized in the expression $y = bx^k$ of HUXLEY, if they are slightly amplified.

Sometime ago NOMURA (1926, '28) studied the local variation in some molluscan shells. He applied an empirical formula $a = kb^x$ to his results, this formula having the same form as HUXLEY's $y = bx^k$ but only with the adoption of a different notation. According to him, his k expresses a local constant and his x a specific constant.

Naturally, the value of the local constant ought to vary with the locality. Variation in the local constant ought to represent the differences in the environment where the animals live. If this proves to be the case, the problem of what environmental factors affect the shell-development is not only an interesting but also a vital one in the field of ecological investigation. The study of this problem was therefore undertaken at the suggestion of Prof. E. NOMURA, and begun on April 1, 1933, *Meretrix meretrix* (L.) being chosen as the material, and the empirical formula $y=ax^b$ being applied, where a and b denote different kinds of constant, and x and y different kinds of variable, each of which represents a series of respective measurements taken from the shells.

Here I wish to express my sincere thanks, to Prof. EKITARO NOMURA, who kindly suggested to me useful subject, and gave me cordial guidance during the progress of my investigation, and also to Mr. SHICHIMEI NOMURA, who willingly identified the species. For collecting the specimens and for having been reported on the conditions of sea-water and sea-bottom, I am also much indebted to the gentlemen: Mr. S. KONDÔ, Mr. R. SATÔ and Mr. Z. KANZAKI of the Fishery Institute, Ehime-Ken; Mr. K. MORIOKA and Mr. D. OKABE of the Fishery Guild, Kamesima, Okayama-Ken; Mr. T. SITANO, the Fishery Guild, Ôzî-Mura, Yamaguti-Ken; Mr. H. SIMURA, Mr. M. KAWAMURA and Mr. KATÔ of the Fishery Institute, Kumamoto-Ken; Mr. G. KUMAGAI, the Fishery Institute, Buzen-Kai, Iukuoka-Ken; Mr. S. MIYAZAKI, the Fishery Institute, Ariake-Kai, Iukuoka-Ken; Mr. K. ISHIKAWA, the Fishery Institute, Hukuoka, Hukuoka-Ken; Mr. M. TAMURA, the Fishery Institute, Hiroshima-Ken, Mr. K. MAEDA, the Fishery Institute, Tottori-Ken; Mr. T. NOGUTI, and Mr. H. MIYATIKI of the Fishery Institute, Tokushima-Ken; and also to the Fishery Institutes of the Prefectures: Ehime-Ken; Kumamoto-Ken; Iukuoka-Ken; Hiroshima-Ken; Tottori-Ken; Tokushima-Ken; Okayama-Ken; Yamaguti-Ken; Iukushima-Ken; Isikawa-Ken; Miyagi-Ken; and Tiba-Ken; and to the Fish-Breeding Station, Kawagoe, Miye-Ken.

MATERIAL AND METHOD

Meretrix meretrix (L.) is a clam widely distributed on the sandy sea-shore of Japan. The materials have been collected from fourteen different places in Honsyû, Sikoku and Kyûsyû, as shown in Fig. 1.

For preserving the specimens, a 3% formalin solution was used. This solution was always kept at a specific gravity of 1.024, with the addition of a quantity of sodium chloride.

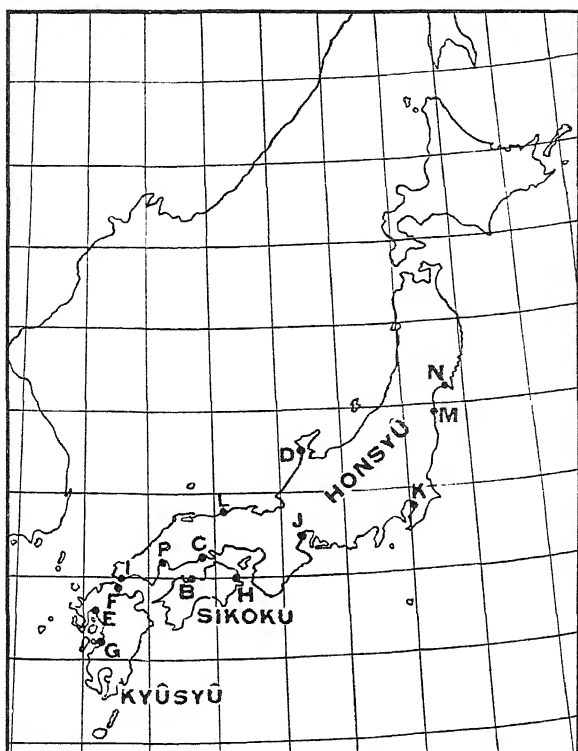


Fig. 1 Main islands of Japan, to show the localities where the clam-shells were collected.

- A and F Yatuya-Mati, Tikuzyô-Gun, Hukuoka-Ken.
- B Saizyô-Mati, Nii-Gun, Ehime-Ken.
- C Turesima-Mati, Asakuti-Gun, Okayama-Ken.
- D Takamatu-Mati, Kahoku-Gun, Isikawa-Ken.
- E Ryôkai-Mura, Sanmon-Gun, Hukuoka-Ken.
- G Yatusiio-Mati, Yatusiio-Gun, Kumamoto-Ken.
- H Tokusima, Tokusima-Ken.
- I Ôzi-Mura, Toyoura-Gun, Yamaguti-Ken.
- J Kawagoe-Mura, Miye-Gun, Miye-Ken.
- K Kankawa, Tiba, Tiba-Ken.
- L Hattori-Mura, Iwami-Gun, Tottori-Ken.
- M Matukawa-Ura, Sôma-Gun, Hukushima-Ken.
- N Watanoha-Mati, Ozika-Gun, Miyagi-Ken.
- P Kusatu-Mati, Hirosima, Hirosima-Ken.

The linear dimensions, viz. length, depth, and height, and the shell-weight, including ligament only, were measured. All the linear measurements have been calculated as far as to two decimal places in cm., and the shell-weight to one decimal place in gm.

The calculations were made by the method of least squares, the results

being expressed in the logarithmic formula, $\log y = \log a + b \log x$.

In the formula, $y = ax^b$, y is taken as representing the depth, height, or weight, while x always represents the length.

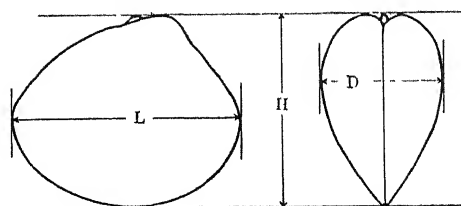


Fig 2 Diagrammatic representation of *Meretrix meretrix*, to show the points where the linear measurements were taken
L length, D depth, H height

RESULTS

In each locality, the date of collection, the shortest and greatest length among the shells obtained, the annual average of the surface temperature of sea-water, and the number of specimens are shown together in Table 1.

TABLE 1.

Place	Date of collection	Shortest and greatest length among the shells obtained (cm)	Average surface temperature (°C)	Number of specimens
A	Nov. 19, '32	4.51—6.42	17.4	38
B	Nov. 20, '32	3.10—5.50	17.7	101
C	Nov. 19, '32	2.76—4.18	--	144
D	Oct. 14, '32	3.71—5.49	--	101
E	Oct. 29, '32	2.02—5.00	17.8	129
F	Nov. 20, '32	2.47—5.37	17.4	114
G	Nov. 4, '32	3.22—4.00	18.6	170
H	Dec. 25, '32	2.66—5.08	17.6	141
I	Jul. 11, '32	2.36—7.70	-	82
J	Sep. 29, '32	2.13—5.59	17.2	181
K	Sep. 30, '32	1.48—5.95	16.4	274
L	Oct. 3, '32	5.80—7.69	--	30
M	Sep. 2, '33	0.52—3.87	14.9	187 ^x
N	Sep. 19, '33	1.17—6.15	14.6	67
P	Feb. 14, '33	2.29—5.16	18.0	184

The notations showing the localities are exactly the same as those in Fig. 1.

* The shell-weights for calculation were taken from 100 individuals, 1.57—3.87 cm. in length.

The results obtained by calculation are shown together in Table 2.

As shown in Table 2, the value of b is never constant. Originally, the constant b is the ratio between two constants and represents a condition of relative growth between two dimensions, x and y . Thus if these two constants vary with locality and season¹, the value of b should naturally be different according to the difference in locality and season (time phase). However, in speaking of one species, the mean value of b can be taken as a representative or standard value for the species. Therefore, in spite of the fact that the value of a , which corresponds directly to the value of b , is not to be preferred as the local constant, the value of a_m , which corresponds to the mean value of b , can be recognized as such a constant. Thus, the local constants are morphologically comparable figures through the whole developmental process, showing the change of the dimension y when the length x is taken as the base.

TABLE 2.

Place	Depth			Height			Weight		
	b	a	a_m	b	a	a_m	b	a	a_m
B	1.00	0.494	0.466	0.95	0.887	0.913	2.68	0.188	0.197
C	1.12	0.431	0.475	0.98	0.840	0.893	2.50	0.227	0.190
D	1.02	0.436	0.422	0.85	0.985	0.868	2.76	0.201	0.239
E	0.99	0.524	0.488	0.95	0.883	0.908	2.73	0.164	0.183
F	1.00	0.524	0.498	0.92	0.934	0.922	2.59	0.226	0.210
G	1.09	0.470	0.499	0.91	0.943	0.921	2.55	0.249	0.221
H	0.98	0.516	0.476	0.90	0.969	0.931	2.56	0.283	0.251
I	0.96	0.549	0.491	0.92	0.941	0.928	2.55	0.260	0.227
J	0.99	0.514	0.483	0.90	0.927	0.894	2.40	0.276	0.203
K	1.03	0.453	0.448	0.92	0.885	0.875	2.66	0.167	0.169
M	1.04	0.459	0.459	0.99	0.861	0.891	2.70	0.242	0.254
N	1.17	0.356	0.420	0.95	0.841	0.862	3.10	0.134	0.237
P	1.08	0.462	0.483	0.90	0.932	0.901	2.74	0.174	0.193
Mean	1.04			0.93			2.65		

The data from the places A and L are omitted because of the small number of the individual specimens at those places.

The value of a corresponds directly to the value of b , but the value of a_m corresponds only to the mean value of b .

¹ The details in connection with this point will be discussed in another paper.

PROBABLE RELATION BETWEEN LOCAL CONSTANTS AND TEMPERATURE

It is very natural to consider that the local constants have some relation to the environment, because the development of the shell ought to be controlled by the environmental system in which the animal grows. In such cases, temperature of sea-water, salinity, nutrition, and many other physical, chemical, and biological factors may be considered as the limiting factors of shell growth. These external factors may accelerate or retard

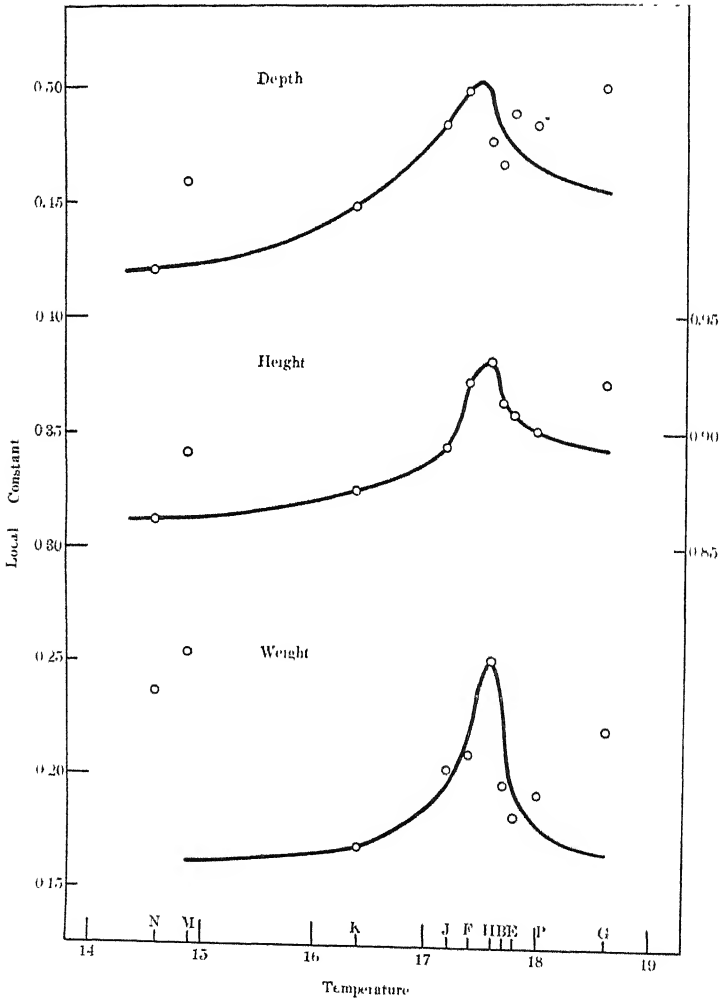


Fig. 3. Relations between the local constants and the annual average of surface temperature of sea-water. Plotted from the data given in Tables 1 and 2.

the shell growth, and also may be expected to be in equilibrium with the internal factors of the animal. Unfortunately, however, the records of many environmental factors which might affect the growth are very meagre with the exception of those of average temperature of the surface of sea-water.

A probable relation between the local constants and the temperature is shown in Fig. 3.

Invariably in the three curves, the maximum local constant is found at about 17.6°C., and the value of the local constant tends to decrease in accordance with both lowering and raising of the temperature. The general shape of the curves is not symmetrical. Some points at both low and high temperature do not exactly fit in with the curves. In my opinion, these points may have been caused by other effective environmental factors, which cannot yet be identified.

The local constant represents, of course, the morphological variation: strictly speaking, the variation in the weight, height and in the depth, when the length is the same. In comparison with the side view of the clams from different localities, the anterior or posterior contour of the shells appears to be different according to the different temperature. In

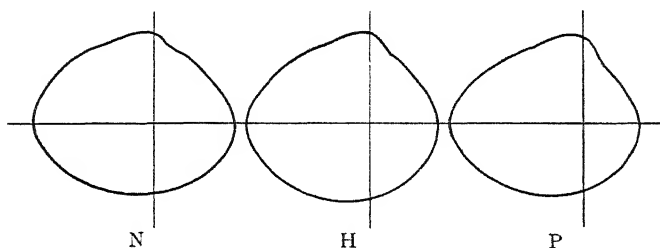


Fig. 4. Diagrammatic side view of the clams from the different localities. N from Watanoha-Mati, Miyagi-Ken (14.6°C), H from Tokusima, Tokusima-Ken (17.6°C), P from Kusatu-Mati, Hiroshima-Ken (18.0°C.)

general, the lower the temperature the more the anterior part of the shell projects, the higher the temperature the more the posterior part projects, and the nearer the temperature approaches 17.6°C. the more rounded the outline of the shell becomes.

DISCUSSION

NOMURA (1926, '28) studied the differences in the local constants in *Littorina sitchana* PHIL., *Sphaerium heterodon* PILS., *Limnaea japonica* JAY,

Viviparus japonicus var. *iwakawai* PILS., *Purpura clavigera* KUSTER, and *Monodonta labio* (LINN.).

CHAMBERLAIN (1930) has shown that the rate of growth of the fresh-water molluscus, *Lampsilis anodontoides* (LEA), *Lampsilis siliquoidea pepinensis* BAKER, and *Tritogonia vercosa* (RAFINESQUE), varies with locality. In these molluscan shells, discrepancy in the local constant may be also recognized as due to difference in the rate of growth in different localities.

In 1926 NOMURA observed the influence of coastal waves on the shell-shape of *Littorina sitchana*. The effect of coastal waves on the material under discussion can be readily determined. At the locality N which is exposed to comparatively high waves, as it faces the outer ocean, the thickness of the valve of the clams is larger than that from the localities facing a calm sea. Moreover, the specimens from the place N are heavier than those from other places, but this may be considered to be due not only to the effect of coastal waves but also to other factors.

In sea-water, oxygen-amount, salinity, and temperature are mutually closely related.

THIEL (1926) has observed in the case of *Sphaerium corneum* that the growth rate of shell depth and height in proportion to length varies with the cleanliness and uncleanness of the water in the locality. He has ascertained, experimentally, that this phenomenon is caused by the difference in the oxygen-content in the medium.

Salinity of sea-water has a very remarkable effect on the shell growth, as well as in calcareous metabolism. ORTON (1925) has studied the shell growth of the oyster and concluded that the growth is accelerated in a low salinity.

In general, temperature is known as an effective factor in the velocity of growth. ORTON (1928) observed that the shell growth of the oyster, *Ostrea edulis*, is most vigorous in spring and autumn, and almost ceases in summer and winter, and he states that the temperature during the shell-growing period is 50°–59°F. This fact may suggest the existence of a correlation between temperature and shell growth. Thus the presence of different constants found in my present research may possibly be considered as one proof that the shell-development shows a close correlation with temperature.

SUMMARY

1) The length, height, depth, and the shell-weight of the clam, *Meretrix meretrix* (L.), from fourteen different places have been measured.

2) In order to determine the local constants, the formula, $y=ax^b$, where x is always the length, y another dimension, and a and b constants, is used. The local constant a_m is calculated out from the formula after the determination of the mean value of b .

3) At an annual average of surface temperature of sea-water of about 17.6°C., the local constant showed the maximum value. The side view of the clams grown at this temperature is comparatively roundish.

4) The lower the temperature the more marked is the anterior projection, and the higher the temperature the more acute the posterior projection becomes.

PUBLICATIONS AND REPORTS

- ANDERSON, B. G. 1932. The Number of Pre-adult Instars, Growth, Relative Growth, and Variation in *Daphnia magna*. Biol. Bull., 63.
- ANDERSON, B. G. 1933. Regeneration in the Carapace of *Daphnia magna*. I. The Regeneration and the Area of the Wound during Single Adult Instars. Biol. Bull., 64.
- CHAMBERLAIN, T. K. 1930. Annual Growth of Fresh-water Mussels. Bull. Bureau Fisheries, U. S. A., 46.
- CROZIER, W. J. 1914. The Growth of the Shell in the Lamellibranch, *Dosinia discus* (REEVE). Zool. Jahrb., Abt. Anat. u. Ont., 38.
- GREEN, C. V. and E. FEKETE. 1933. Differential Growth in the Mouse. Jour. Exper. Zool., 66.
- HERSH, A. H. 1931. Facet Number and Genetic Growth Constants in Bar-eyed Stocks of *Drosophila*. Jour. Exper. Zool., 60.
- HUXLEY, J. 1927. Further Work on Heterogonic Growth. Biol. Zentbl., 47, Heft 3.
- HUXLEY, J. 1931. Notes on Differential Growth. Amer. Nat., 65.
- HUXLEY, J. 1931. Relative Growth of Mandibles in Stag-beetles (*Lucanidae*). Linn. Soc. Jour. Zool., 37. No. 255.
- HUXLEY, J. 1931. Problems of Relative Growth. London.
- HUXLEY, J. and RICHARDS, O. W. 1931. Relative Growth of the Shore-Crab, *Caracinus maenas*. Jour. Mar. Biol. Assoc., 17.
- JANISCH, E. 1927. Das Exponentialgesetz als Grundlage einer vergleichenden Biologie. Berlin.
- KANITZ, A. 1915. Temperatur und Lebensvorgänge. Berlin.
- KLEIN, A. D. and SCAMMON, R. E. 1930. Relation between Surface Area, Weight and Length of the Human Body in Prenatal Life. Proc. Soc. Exper. Biol. and Med., 27.
- KLEIN, A. D. and SCAMMON, R. E. 1930. The Regional Growth in Surface Area of the Human Body in Prenatal Life. Proc. Soc. Exper. Biol. and Med., 27.
- NOMURA, E. 1926. The Influence of Coastal Waves on the Shell-development in *Littorina sitchana* PHIL. Science Reports Tōhoku Imp. Univ., Biology, 2.
- NOMURA, E. 1926. An Application of $a=kb^x$ in Expressing the Growth Relation in the Freshwater Bivalve, *Sphaerium heterodon* PILS. Science Reports Tōhoku Imp. Univ., Biology, 2.

- NOMURA, E. 1926. Further Studies on the Applicability of $a=kb'$ in Expressing the Growth Relations in Molluscan Shells. Science Reports Tōhoku Imp Univ, Biology, 2
- NOMURA, E. 1928. On the Local Variation in some Littoral Gastropods. Science Reports Tōhoku Imp. Univ, Biology, 3.
- NOMURA, SHICHIHEI 1933. Catalogue of the Tertiary and Quaternary Mollusca from the Island of Taiwan (Formosa) in the Institute of Geology and Paleontology, Tōhoku Imperial University, Sendai, Japan Part I. Pelecypoda. Science Reports Tōhoku Imp. Univ, Geology, 16.
- ORTON, J. H. 1925. The Conditions for Calcareous Metabolism in Oysters and Marine Animals. Nature, 116.
- ORTON, J. H. 1928. On Rhythmic Periods in Shell-growth in *Ostrea edulis* with a Note on Fattening. Jour. Mar. Biol. Assoc, 15, No. 2.
- PRZIBRAM, H. 1922. Form und Formel im Tierreiche Leipzig u Wien
- PRZIBRAM, H. 1923. Temperatur und Temperaturen im Tierreiche Leipzig u Wien
- SCAMMON, R. E. 1930. The Ponderal Growth of the Extremities of the Human Fetus Proc. Soc. Exper. Biol. and Med, 27.
- THIEL, M. E. 1926. Formwachstumsversuche an *Sphaerium corneum*. Arch. Ent.-mech., 108.
- THOMPSON, D'ARCY, W. 1917 Growth and Form Cambridge

TABLES AS THE BASES OF CALCULATION

TABLE 3. Locality A: Yatuya-Mati, Hukuoka-Ken.
Collected on Nov. 20, 1932.

Length in cm.	Depth in cm.	Height in cm	Weight in gm.	Length in cm.	Depth in cm	Height in cm.	Weight in gm
4.51	2.33	3.73	11.0	5.62	2.98	4.60	23.5
4.59	2.55	3.71	11.7	5.65	2.99	4.40	23.0
5.00	2.70	4.06	16.7	5.70	2.92	4.84	21.8
5.09	2.72	4.12	15.7	5.72	3.10	4.66	24.6
5.23	2.83	4.27	19.9	5.73	3.04	4.53	24.4
5.26	2.82	4.34	14.6	5.75	2.95	4.73	25.3
5.27	2.87	4.25	20.7	5.75	3.01	4.66	21.5
5.29	2.83	4.25	17.6	5.78	2.91	4.61	19.8
5.30	2.78	4.15	17.4	5.82	2.99	4.62	21.6
5.33	2.83	4.31	20.1	5.87	3.00	4.86	22.3
5.36	2.94	4.38	22.2	5.89	3.07	4.77	24.3
5.48	2.81	4.45	18.0	5.90	2.96	4.63	22.9
5.52	3.00	4.56	22.7	5.96	3.10	4.67	26.0
5.52	2.95	4.55	19.5	5.97	3.05	4.72	25.7
5.55	2.88	4.40	20.3	5.97	3.06	4.64	24.5
5.56	2.92	4.49	20.5	6.02	3.06	4.80	24.6
5.57	2.83	4.46	21.9	6.02	3.12	4.79	25.1
5.59	2.89	4.40	19.3	6.10	3.22	4.95	28.2
5.60	2.83	4.52	20.7	6.42	3.17	5.20	26.7

TABLE 4. Locality B: Saizyô-Mati, Ehme-Ken.
Collected on Nov. 20, 1932.

Length in cm.	Depth in cm	Height in cm	Weight in gm.	Length in cm.	Depth in cm	Height in cm.	Weight in gm
3.10	1.49	2.15	3.9	4.15	1.92	3.42	8.8
3.16	1.51	2.66	3.8	4.15	2.25	3.46	8.7
3.32	1.62	2.84	5.1	4.17	2.04	3.47	8.8
3.40	1.65	2.81	4.0	4.17	2.06	3.44	7.8
3.41	1.73	2.88	5.8	4.17	2.03	3.47	9.8
3.61	1.76	3.04	6.1	4.20	2.17	3.38	8.3
3.64	1.80	3.08	5.0	4.21	2.12	3.53	10.2
3.65	1.79	3.03	6.1	4.21	2.08	3.44	9.0
3.65	1.85	3.19	6.3	4.25	2.06	3.57	9.4
3.68	1.90	3.22	6.7	4.25	2.12	3.61	11.0
3.68	1.76	3.07	5.7	4.25	2.13	3.57	10.4
3.71	1.87	3.06	7.2	4.27	2.27	3.53	10.5
3.75	1.87	3.19	6.5	4.31	2.03	3.69	9.9
3.76	1.92	3.21	7.1	4.31	2.09	3.39	8.0
3.78	1.85	3.18	6.8	4.31	2.31	3.63	11.4
3.79	1.94	3.10	6.6	4.31	2.12	3.38	8.0
3.80	1.90	3.15	7.1	4.32	2.08	3.61	9.8
3.81	1.80	3.21	7.0	4.33	2.17	4.03	9.5
3.82	1.97	3.15	6.8	4.38	2.13	3.48	10.4
3.85	1.80	3.24	7.7	4.39	2.27	3.54	10.1
3.86	1.88	3.19	6.1	4.40	2.24	3.69	9.0
3.87	1.90	3.18	6.2	4.41	2.18	3.67	12.7
3.88	1.84	3.14	7.4	4.50	2.16	3.72	9.6
3.88	1.91	3.25	6.8	4.50	2.24	3.68	12.0
3.90	1.89	3.27	6.4	4.52	2.10	3.71	10.1
3.91	1.90	3.25	8.2	4.54	2.23	3.71	11.2
3.93	1.99	3.20	7.5	4.54	2.24	3.80	9.8
3.93	1.97	3.22	7.5	4.55	2.09	3.65	10.7
3.94	1.89	3.13	6.6	4.58	2.37	3.72	10.9
3.95	1.86	3.39	7.4	4.58	2.35	3.80	12.1
3.95	1.91	3.26	7.7	4.65	2.37	3.77	13.1
3.96	1.94	3.20	6.6	4.68	2.32	3.86	12.1
3.96	1.84	3.23	7.8	4.78	2.41	3.88	11.5
3.97	1.93	3.34	6.5	4.80	2.47	3.96	13.1
3.98	1.93	3.38	7.8	4.80	2.23	3.86	11.7
4.02	1.91	3.33	8.7	4.83	2.44	4.03	13.7
4.03	1.98	3.29	7.2	4.90	2.40	3.90	11.9
4.04	2.03	3.37	8.4	4.90	2.30	3.94	12.3
4.04	2.05	3.37	7.3	4.90	2.43	4.02	16.0
4.05	2.04	3.34	7.1	4.91	2.60	4.17	14.6
4.05	2.05	3.37	7.8	4.91	2.49	3.99	12.8
4.05	1.95	3.24	7.2	4.92	2.50	4.13	11.7
4.06	2.08	3.33	8.2	4.97	2.41	4.03	14.3
4.08	2.03	3.29	7.8	4.98	2.55	4.03	13.3
4.09	2.05	3.33	8.9	5.07	2.47	4.23	15.8
4.11	2.03	3.38	8.5	5.10	2.38	4.05	12.9
4.12	2.10	3.32	8.0	5.13	2.40	4.14	12.3
4.12	2.06	3.52	9.7	5.17	2.44	4.11	14.2
4.15	2.15	3.57	8.5	5.30	2.67	4.24	20.3
4.15	2.04	3.43	8.2	5.50	2.60	4.43	17.6
4.15	2.06	3.39	8.9				

TABLE 5. Locality C: Turesima-Mati, Okayama-Ken.
Collected on Nov. 19, 1932.

Length in cm	Depth in cm.	Height in cm	Weight in gm	Length in cm	Depth in cm	Height in cm.	Weight in gm.
2.76	1.37	2.21	2.8	3.24	1.60	2.72	4.5
2.80	1.31	2.29	2.8	3.24	1.62	2.56	4.1
2.81	1.36	2.37	3.3	3.25	1.56	2.60	4.1
2.81	1.36	2.33	2.8	3.25	1.63	2.70	4.2
2.85	1.46	2.36	3.5	3.25	1.62	2.70	4.7
2.87	1.43	2.35	3.3	3.28	1.67	2.66	4.5
2.89	1.35	2.36	3.2	3.29	1.70	2.68	4.3
2.89	1.44	2.52	3.0	3.30	1.68	2.68	4.2
2.91	1.47	2.41	3.7	3.30	1.66	2.67	4.1
2.92	1.45	2.42	3.3	3.30	1.65	2.70	4.3
2.92	1.37	2.34	3.1	3.30	1.60	2.73	4.1
2.92	1.41	2.39	3.4	3.32	1.62	2.81	4.8
2.92	1.41	2.40	3.0	3.32	1.72	2.72	5.7
2.95	1.36	2.42	3.3	3.34	1.69	2.70	4.3
2.97	1.49	2.43	3.6	3.34	1.70	2.71	4.7
2.99	1.40	2.42	3.5	3.34	1.59	2.69	4.2
3.00	1.49	2.40	3.5	3.34	1.69	2.74	4.6
3.00	1.49	2.50	3.6	3.35	1.69	2.79	4.8
3.01	1.43	2.43	3.3	3.36	1.62	2.72	4.5
3.01	1.44	2.40	3.6	3.37	1.76	2.84	5.5
3.01	1.44	2.44	3.6	3.38	1.58	2.78	4.7
3.02	1.50	2.50	3.3	3.38	1.71	2.82	5.4
3.02	1.45	2.48	3.7	3.38	1.71	2.72	4.9
3.03	1.55	2.53	3.9	3.40	1.73	2.74	4.6
3.04	1.53	2.55	4.0	3.40	1.72	2.78	4.7
3.05	1.45	2.52	3.5	3.41	1.74	2.77	5.4
3.05	1.53	2.47	3.6	3.42	1.71	2.85	4.9
3.06	1.52	2.58	3.8	3.42	1.70	2.87	5.0
3.07	1.51	2.49	4.0	3.43	1.74	2.85	4.8
3.07	1.50	2.56	3.6	3.44	1.79	2.75	4.5
3.07	1.52	2.51	3.7	3.45	1.74	2.91	6.0
3.08	1.53	2.47	3.9	3.46	1.78	2.81	4.6
3.08	1.53	2.52	3.7	3.46	1.75	2.91	5.2
3.10	1.56	2.60	4.1	3.47	1.69	2.83	4.4
3.10	1.57	2.58	4.0	3.48	1.69	2.85	4.8
3.10	1.68	2.60	4.3	3.48	1.81	2.92	5.5
3.11	1.46	2.59	3.6	3.48	1.74	2.77	5.2
3.11	1.53	2.55	3.8	3.49	1.70	2.89	5.8
3.12	1.58	2.60	4.1	3.50	1.70	2.85	5.3
3.13	1.49	2.54	3.6	3.50	1.69	2.75	4.9
3.14	1.57	2.62	4.2	3.51	1.72	2.97	5.1
3.15	1.54	2.57	4.0	3.51	1.72	2.86	5.8
3.15	1.48	2.54	3.7	3.51	1.75	2.98	5.7
3.17	1.59	2.63	3.9	3.52	1.84	2.87	5.8
3.17	1.53	2.58	4.0	3.53	1.76	2.91	5.3
3.18	1.60	2.59	4.0	3.53	1.84	2.90	5.3
3.19	1.62	2.63	4.4	3.53	1.68	2.80	5.2
3.19	1.55	2.57	4.0	3.55	1.75	2.89	5.6
3.19	1.72	2.64	4.9	3.55	1.70	2.91	5.0
3.20	1.52	2.65	4.1	3.56	1.86	2.95	5.8
3.20	1.59	2.62	4.1	3.58	1.69	2.86	5.4
3.20	1.62	2.60	4.0	3.58	1.80	2.88	5.2
3.22	1.75	2.63	4.4	3.61	1.84	2.97	6.4
3.22	1.66	2.67	5.2	3.63	1.86	3.02	5.5
3.23	1.61	2.61	4.1	3.63	1.89	3.05	5.5

3.64	1.78	3.06	5.9	3.83	1.90	3.11	7.2
3.66	1.90	3.02	5.8	3.84	2.11	3.15	7.4
3.68	1.87	2.99	6.6	3.84	1.91	3.16	6.4
3.68	1.81	3.00	5.9	3.85	2.03	3.20	7.5
3.69	1.88	2.96	5.5	3.86	1.96	3.30	7.0
3.71	1.85	3.04	6.4	3.87	1.90	3.10	6.2
3.71	1.92	3.08	6.1	3.89	1.94	3.18	7.0
3.72	1.88	3.06	5.6	3.90	1.98	3.31	7.9
3.73	1.84	3.08	6.0	3.91	1.91	3.19	7.5
3.73	1.78	3.02	5.6	3.92	2.03	3.23	6.9
3.74	1.95	3.03	6.3	3.93	2.03	3.20	6.7
3.77	2.06	3.18	5.9	3.95	2.05	3.23	6.4
3.78	1.96	3.06	6.7	3.97	1.99	3.22	7.1
3.79	1.96	3.11	6.1	3.98	2.03	3.23	8.0
3.80	1.84	3.10	6.1	4.01	1.98	3.10	6.0
3.81	1.81	2.97	5.8	4.13	2.14	3.37	7.1
3.81	1.84	3.14	5.0	4.18	2.17	3.39	7.9

TABLE 6. Locality D: Takamatu-Mati, Isikawa-Ken.
Collected on Oct. 14, 1932.

Length in cm	Depth in cm	Height in cm	Weight in gm	Length in cm	Depth in cm	Height in cm	Weight in gm.
3.71	1.65	3.03	7.6	4.79	2.18	3.71	14.4
4.05	1.86	3.25	9.8	4.79	2.15	3.62	15.2
4.30	1.98	3.44	11.1	4.79	2.29	3.70	15.7
4.35	1.95	3.41	11.8	4.81	2.08	3.72	15.1
4.35	1.99	3.35	11.0	4.81	2.28	3.83	16.6
4.41	2.11	3.61	13.7	4.85	2.29	3.76	16.8
4.43	1.97	3.43	12.0	4.85	2.17	3.81	15.3
4.47	1.96	3.45	11.8	4.85	2.34	3.77	17.4
4.49	1.94	3.45	12.4	4.85	2.16	3.76	15.6
4.51	2.04	3.65	12.7	4.85	2.15	3.82	15.8
4.51	2.08	3.56	13.5	4.87	2.11	3.62	14.3
4.52	1.98	3.44	11.6	4.88	2.32	3.77	16.6
4.52	2.00	3.48	12.0	4.88	2.27	3.93	18.2
4.52	2.03	3.53	12.7	4.90	2.14	3.83	15.7
4.53	2.00	3.53	13.4	4.90	2.27	3.72	16.1
4.54	2.01	3.62	13.2	4.90	2.32	3.86	18.1
4.59	2.00	3.53	13.0	4.92	2.33	3.84	18.3
4.60	2.04	3.62	13.5	4.94	2.20	3.91	15.6
4.60	1.93	3.54	12.7	4.95	2.20	3.80	16.7
4.61	1.90	3.60	12.1	4.96	2.30	3.85	16.7
4.61	2.05	3.62	13.5	4.97	2.21	3.86	17.3
4.62	2.08	3.63	13.2	4.97	2.29	3.80	16.6
4.64	2.03	3.66	13.2	4.98	2.19	3.88	15.4
4.65	2.17	3.75	15.2	4.98	2.20	3.78	16.1
4.65	2.06	3.68	13.9	4.99	2.20	3.88	16.5
4.66	2.01	3.60	12.9	4.99	2.20	3.73	16.2
4.69	2.04	3.73	14.3	5.00	2.21	3.95	18.0
4.71	2.12	3.62	14.0	5.00	2.31	3.91	18.5
4.71	2.08	3.72	14.3	5.01	2.29	3.86	17.0
4.72	2.18	3.67	15.1	5.01	2.27	3.90	16.7
4.73	2.08	3.70	14.4	5.01	2.24	3.89	16.9
4.73	2.10	3.62	14.3	5.01	2.26	3.94	18.4
4.76	2.16	3.78	15.3	5.02	2.26	3.97	19.0
4.77	2.31	3.86	16.2	5.02	2.16	3.90	17.1
4.78	2.12	3.67	14.6	5.02	2.32	3.92	17.0

5.02	2.38	3.94	17.8	5.17	2.50	4.07	21.1
5.03	2.25	3.80	15.5	5.18	2.41	4.14	21.1
5.03	2.28	4.06	17.7	5.21	2.26	3.90	18.2
5.06	2.13	3.81	16.0	5.22	2.45	3.94	19.4
5.06	2.21	3.89	17.0	5.25	2.30	4.01	19.2
5.08	2.36	3.94	18.2	5.26	2.30	3.93	17.9
5.09	2.42	3.92	18.7	5.30	2.39	3.95	18.9
5.09	2.24	3.92	17.9	5.31	2.40	4.16	20.8
5.10	2.25	3.86	18.2	5.33	2.46	4.08	20.2
5.10	2.29	4.00	17.2	5.33	2.29	4.00	18.6
5.10	2.29	3.96	18.0	5.34	2.40	4.09	21.3
5.11	2.30	3.83	17.1	5.39	2.40	4.05	19.5
5.12	2.35	4.01	18.5	5.41	2.56	4.26	23.0
5.12	2.33	3.91	18.3	5.43	2.41	4.11	20.5
5.13	2.31	3.99	18.7	5.45	2.41	4.19	21.4
5.15	2.38	4.05	18.9	5.48	2.35	4.16	20.8
5.16	2.30	4.13	20.0	5.49	2.35	4.08	21.3

TABLE 7. Locality E: Ryôkai-Mura, Hukuoka-Ken
Collected on Oct. 29, 1932.

Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Length in cm.	Depth in cm.	Height in cm.	Weight in gm.
2.02	0.99	1.70	1.2	3.84	1.90	3.21	6.9
3.23	1.61	2.70	3.7	3.84	2.00	3.17	6.1
3.39	1.66	2.90	4.2	3.85	1.97	3.12	6.5
3.40	1.85	2.84	5.7	3.86	1.98	3.17	6.0
3.48	1.73	2.84	4.3	3.88	1.98	3.23	7.1
3.53	1.79	2.98	4.1	3.88	2.01	3.18	5.9
3.53	1.84	2.93	5.1	3.89	2.09	3.17	6.2
3.55	1.89	2.95	5.4	3.90	2.12	3.27	8.2
3.61	1.83	3.02	5.7	3.90	2.08	3.21	7.2
3.62	1.93	2.97	5.6	3.90	2.10	3.22	7.3
3.62	1.90	3.00	5.5	3.90	1.97	3.24	6.8
3.63	1.89	2.90	4.8	3.92	2.01	3.19	7.0
3.64	1.85	2.88	4.9	3.92	2.02	3.14	6.5
3.66	1.94	3.01	6.0	3.93	1.99	3.20	6.7
3.67	1.95	3.01	5.3	3.93	2.15	3.28	7.7
3.72	1.97	3.12	5.7	3.94	1.96	3.25	6.6
3.72	1.96	3.06	6.1	3.95	2.00	3.13	6.4
3.72	2.04	3.05	5.7	3.98	2.10	3.13	6.6
3.73	1.86	3.12	5.2	3.98	2.03	3.29	7.0
3.74	1.89	3.10	5.8	3.99	2.00	3.35	7.9
3.74	2.04	3.13	7.3	4.00	1.89	3.26	5.7
3.76	1.98	3.21	5.7	4.01	1.99	3.23	6.0
3.76	2.07	3.06	6.3	4.01	2.11	3.30	8.3
3.77	1.98	3.19	6.3	4.01	2.01	3.25	8.0
3.77	1.98	3.10	5.9	4.02	2.11	3.43	8.1
3.78	1.89	3.15	5.7	4.02	2.10	3.33	7.5
3.78	1.93	3.15	6.6	4.03	2.06	3.25	6.9
3.80	2.09	3.08	6.6	4.04	2.08	3.37	7.0
3.80	1.95	3.09	5.9	4.04	2.05	3.35	7.1
3.80	2.01	3.21	6.5	4.05	2.13	3.37	7.7
3.81	2.02	3.20	7.7	4.05	2.07	3.35	8.1
3.81	2.05	3.23	6.9	4.05	2.21	3.35	7.4
3.82	1.93	3.14	6.2	4.07	2.14	3.43	8.0
3.82	1.97	3.26	6.6	4.07	2.10	3.34	7.1
3.83	1.96	3.12	5.6	4.09	2.17	3.42	7.7

4.09	2.12	3.37	6.8	4.37	2.20	3.73	9.9
4.09	2.10	3.27	7.1	4.37	2.14	3.62	8.7
4.10	2.28	3.38	8.9	4.38	2.27	3.56	9.6
4.11	2.19	3.52	8.7	4.39	2.17	3.48	7.8
4.11	2.12	3.43	8.6	4.40	2.22	3.59	9.0
4.12	2.03	3.39	6.6	4.41	2.25	3.65	9.9
4.14	2.20	3.47	9.1	4.41	2.25	3.58	8.5
4.14	2.19	3.45	8.7	4.42	2.37	3.50	10.4
4.14	2.29	3.43	7.9	4.43	2.27	3.72	9.9
4.14	2.07	3.43	7.0	4.46	2.22	3.65	8.0
4.16	2.18	3.38	7.9	4.47	2.38	3.71	10.3
4.16	2.16	3.32	8.0	4.47	2.20	3.58	9.8
4.18	2.17	3.53	8.6	4.48	2.31	3.70	10.1
4.19	2.21	3.45	8.2	4.49	2.23	3.64	8.8
4.19	2.15	3.52	9.0	4.49	2.30	3.62	9.0
4.19	2.25	3.39	8.9	4.50	2.25	3.68	9.7
4.20	2.13	3.39	7.7	4.52	2.39	3.69	10.4
4.20	2.05	3.49	8.8	4.52	2.23	3.73	10.0
4.21	2.20	3.36	7.9	4.53	2.29	3.58	9.4
4.23	2.20	3.44	8.0	4.53	2.29	3.74	10.8
4.24	2.26	3.60	8.8	4.64	2.40	3.67	11.1
4.25	2.23	3.61	9.8	4.65	2.49	3.78	11.0
4.27	2.21	3.57	8.6	4.65	2.46	3.80	12.3
4.28	2.20	3.50	9.9	4.67	2.42	3.81	9.7
4.32	2.22	3.54	7.8	4.79	2.44	3.85	13.1
4.32	2.12	3.54	8.8	4.84	2.45	3.99	12.9
4.33	2.11	3.57	8.8	4.87	2.50	3.99	11.2
4.34	2.09	3.62	8.4	4.92	2.40	4.02	12.2
4.34	2.27	3.63	10.1	5.00	2.56	4.05	14.8
4.36	2.18	3.56	8.6				

TABLE 8. Locality F: Yatuya-Mati, Hukuoka-Ken.
Collected on Nov. 19, 1932.

Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Length in cm.	Depth in cm.	Height in cm.	Weight in gm.
2.47	1.25	2.11	2.2	3.15	1.62	2.67	4.2
2.63	1.30	2.27	2.6	3.15	1.72	2.70	4.7
2.63	1.42	2.30	3.1	3.20	1.66	2.72	5.2
2.74	1.45	2.34	3.2	3.21	1.67	2.75	4.3
2.77	1.37	2.31	2.8	3.21	1.55	2.71	3.8
2.77	1.49	2.42	3.3	3.21	1.77	2.70	4.8
2.80	1.46	2.39	2.9	3.22	1.71	2.72	4.8
2.89	1.55	2.54	3.7	3.23	1.62	2.76	4.9
2.90	1.52	2.45	3.2	3.24	1.89	2.84	5.8
2.92	1.58	2.51	4.0	3.26	1.64	2.75	4.6
2.92	1.59	2.52	4.1	3.30	1.62	2.71	4.2
2.93	1.62	2.50	4.1	3.30	1.79	2.83	5.9
3.02	1.56	2.54	3.9	3.32	1.79	2.88	5.7
3.03	1.52	2.51	3.9	3.33	1.68	2.81	5.3
3.03	1.58	2.59	3.5	3.34	1.72	2.83	4.9
3.03	1.65	2.56	4.2	3.35	1.70	2.89	5.6
3.07	1.53	2.69	4.1	3.35	1.85	2.80	6.2
3.08	1.51	2.65	3.1	3.37	1.70	2.78	4.8
3.08	1.58	2.61	4.0	3.37	1.85	2.88	6.5
3.10	1.59	2.63	4.1	3.40	1.76	2.93	5.2
3.11	1.64	2.76	4.8	3.40	1.80	2.89	5.7
3.14	1.57	2.63	3.4	3.41	1.71	2.90	5.6

3.44	1.80	2.95	4.9	3.92	2.00	3.30	8.8
3.45	1.88	2.96	6.2	3.92	2.12	3.29	7.8
3.46	1.90	3.00	6.9	3.93	2.02	3.30	9.5
3.47	1.84	2.92	6.1	3.93	2.10	3.27	8.7
3.53	1.93	3.05	5.0	3.95	2.07	3.30	8.3
3.55	1.96	3.03	6.7	3.95	2.10	3.33	8.2
3.56	1.80	2.91	5.1	4.02	2.07	3.40	8.2
3.58	1.94	3.00	6.2	4.03	2.14	3.43	8.3
3.59	1.80	2.98	5.7	4.04	2.16	3.44	10.8
3.60	1.95	3.06	7.2	4.06	2.18	3.42	10.2
3.62	1.81	3.04	6.7	4.07	2.19	3.44	8.3
3.62	1.88	3.02	6.0	4.07	2.32	3.43	9.5
3.64	1.93	3.01	6.2	4.10	2.24	3.47	10.4
3.65	1.90	3.02	6.6	4.14	2.16	3.35	7.2
3.65	2.08	3.05	6.8	4.14	2.25	3.57	9.6
3.68	1.87	2.99	5.6	4.15	2.20	3.45	8.0
3.68	1.90	3.02	5.6	4.16	2.06	3.45	8.5
3.68	1.94	3.07	5.8	4.16	2.17	3.47	9.5
3.70	2.03	3.11	6.2	4.19	2.11	3.52	9.7
3.70	1.94	3.03	7.1	4.19	2.23	3.48	9.2
3.71	2.05	3.16	6.8	4.20	2.10	3.44	9.3
3.73	1.85	3.16	5.6	4.23	2.21	3.66	9.2
3.73	2.00	3.18	7.6	4.25	2.14	3.60	10.3
3.76	1.99	3.22	7.9	4.31	2.25	3.61	9.4
3.77	2.00	3.12	5.3	4.34	2.22	3.65	9.2
3.79	1.98	3.17	7.7	4.36	2.20	3.41	7.7
3.82	2.00	3.20	8.4	4.40	2.34	3.68	10.2
3.83	1.97	3.21	7.9	4.48	2.32	3.68	11.5
3.83	2.13	3.20	7.9	4.55	2.31	3.75	10.8
3.86	2.05	3.30	7.5	4.56	2.34	3.75	10.3
3.88	2.01	3.22	7.0	4.70	2.46	3.83	12.7
3.90	2.09	3.26	9.0	4.70	2.59	3.91	12.0
3.91	1.97	3.29	6.6	5.05	2.66	4.06	15.7
3.91	2.06	3.28	8.2	5.10	2.72	4.06	13.4
3.92	1.97	3.30	7.3	5.37	2.60	4.38	18.9

TABLE 9. Locality G : Yatusiro-Mati, Kumamoto-Ken.
Collected on Nov. 4, 1932.

Length in cm.	Depth in cm	Height in cm	Weight in gm.	Length in cm	Depth in cm.	Height in cm	Weight in gm
2.03	1.00	1.80	1.4	2.73	1.37	2.30	3.1
2.38	1.19	2.02	2.1	2.74	1.40	2.36	3.4
2.44	1.23	2.06	2.3	2.75	1.42	2.41	3.1
2.51	1.19	2.16	2.4	2.76	1.38	2.33	3.0
2.52	1.29	2.15	2.5	2.77	1.41	2.39	3.4
2.53	1.22	2.13	2.3	2.80	1.44	2.39	3.4
2.56	1.28	2.14	2.6	2.81	1.41	2.39	3.4
2.58	1.32	2.20	2.7	2.83	1.49	2.41	3.9
2.62	1.48	2.33	3.5	2.83	1.53	2.44	3.8
2.63	1.37	2.30	3.0	2.85	1.48	2.51	3.8
2.64	1.42	2.32	3.0	2.86	1.46	2.43	3.9
2.66	1.33	2.24	2.9	2.88	1.48	2.46	3.7
2.66	1.37	2.30	3.0	2.89	1.52	2.51	4.0
2.69	1.41	2.34	3.0	2.90	1.49	2.47	3.3
2.70	1.40	2.31	3.4	2.90	1.55	2.48	3.6
2.70	1.41	2.35	3.6	2.90	1.59	2.51	4.1
2.70	1.41	2.38	3.4	2.91	1.49	2.48	3.8

Length in cm.	Depth in cm.	Height in cm	Weight in gm	Length in cm.	Depth in cm	Height in cm	Weight in gm
2.92	1.51	2.48	3.8	3.40	1.72	2.92	6.1
2.93	1.54	2.53	4.7	3.40	1.79	2.85	6.1
2.93	1.63	2.58	4.2	3.42	1.80	2.91	5.5
2.94	1.45	2.54	4.0	3.44	1.80	2.97	5.7
2.94	1.53	2.56	3.9	3.44	1.90	2.97	6.3
2.95	1.51	2.50	3.6	3.45	1.78	3.04	7.7
2.97	1.54	2.53	4.1	3.45	1.79	2.98	5.9
2.98	1.53	2.37	3.3	3.45	1.81	2.88	4.9
2.98	1.55	2.51	4.4	3.46	1.89	2.90	5.3
3.00	1.48	2.55	4.3	3.46	1.97	2.98	7.2
3.00	1.56	2.58	4.1	3.47	1.84	2.86	5.5
3.00	1.59	2.50	4.0	3.48	1.83	2.91	5.6
3.01	1.57	2.68	4.8	3.48	1.81	2.87	5.2
3.02	1.52	2.62	4.1	3.49	1.83	3.01	5.6
3.04	1.61	2.62	4.6	3.50	1.84	3.01	7.0
3.05	1.55	2.58	4.6	3.50	1.85	2.99	6.1
3.08	1.65	2.69	5.2	3.51	1.82	2.96	6.5
3.09	1.58	2.66	4.2	3.52	1.85	2.95	5.8
3.09	1.64	2.61	4.9	3.52	2.00	3.05	6.8
3.10	1.54	2.60	3.6	3.53	1.85	2.98	6.9
3.10	1.55	2.73	4.7	3.53	1.87	2.97	6.2
3.10	1.65	2.67	5.0	3.53	1.90	2.96	5.8
3.10	1.66	2.70	4.8	3.54	1.85	2.96	5.8
3.11	1.63	2.62	4.0	3.54	1.85	2.96	5.7
3.11	1.67	2.71	4.8	3.54	1.92	2.97	5.8
3.12	1.63	2.61	4.7	3.55	1.82	3.00	6.6
3.13	1.74	2.78	5.0	3.55	1.88	3.00	6.2
3.14	1.60	2.55	4.3	3.57	1.85	2.95	5.4
3.15	1.59	2.71	4.7	3.58	1.89	2.96	5.0
3.15	1.64	2.66	4.5	3.60	1.83	3.00	6.9
3.16	1.60	2.66	4.6	3.60	1.83	3.06	6.7
3.18	1.66	2.72	4.3	3.60	1.89	2.98	6.8
3.19	1.62	2.74	4.9	3.60	1.90	3.06	6.9
3.19	1.62	2.78	4.7	3.60	1.93	2.91	6.6
3.20	1.68	2.74	4.8	3.61	1.81	2.92	5.7
3.21	1.66	2.79	5.6	3.61	1.92	3.05	7.4
3.22	1.66	2.67	4.5	3.61	1.95	3.05	7.3
3.24	1.71	2.83	5.9	3.62	1.76	3.02	7.0
3.24	1.84	2.73	6.0	3.62	1.88	3.07	7.1
3.25	1.66	2.69	4.0	3.63	1.86	2.97	6.0
3.25	1.73	2.75	5.3	3.63	1.90	3.02	6.9
3.25	1.74	2.63	4.2	3.63	1.91	2.99	7.5
3.27	1.66	2.82	4.6	3.63	1.95	3.00	6.4
3.29	1.74	2.80	5.0	3.64	1.94	3.11	7.5
3.29	1.75	2.76	5.2	3.66	1.94	3.06	6.1
3.29	1.76	2.76	5.0	3.67	2.00	3.05	6.0
3.29	1.85	2.81	5.7	3.68	1.83	3.07	6.3
3.30	1.66	2.80	4.3	3.70	1.87	3.15	8.0
3.30	1.74	2.82	5.0	3.70	1.88	3.03	5.9
3.32	1.65	2.80	5.4	3.71	2.04	3.04	7.4
3.33	1.81	2.85	5.5	3.72	1.92	3.07	7.6
3.34	1.71	2.83	5.7	3.72	1.99	3.17	6.9
3.35	1.78	2.86	4.4	3.72	2.05	3.16	8.5
3.35	1.79	2.84	4.8	3.73	1.97	3.22	7.9
3.36	1.73	2.90	5.4	3.74	1.90	3.05	6.7
3.36	1.78	2.82	4.9	3.76	1.92	3.10	6.4
3.37	1.79	2.81	5.6	3.76	1.96	3.12	7.0
3.39	1.72	2.86	5.1	3.77	2.03	3.26	8.3
3.39	1.85	2.91	6.1	3.77	1.97	3.10	6.9

3.78	1.97	3.22	8.2	3.88	2.20	3.16	8.2
3.78	1.98	3.21	7.0	3.90	2.04	3.36	8.6
3.78	2.07	3.11	8.1	3.95	1.94	3.25	7.6
3.80	2.04	3.12	7.8	3.95	2.09	3.24	8.2
3.83	2.00	3.15	6.6	3.96	2.10	3.25	8.6
3.83	2.03	3.33	7.1	3.96	2.11	3.33	9.8
3.83	2.08	3.26	8.8	3.96	2.13	3.26	7.7
3.83	2.09	3.15	6.9	4.00	2.05	3.20	6.8
3.85	2.15	3.25	8.4	4.00	2.17	3.36	9.4

TABLE 10. Locality H: Tokusima, Tokusima-Ken.
Collected on Dec. 25, 1932.

Length in cm.	Depth in cm	Height in cm	Weight in gm	Length in cm	Depth in cm.	Height in cm	Weight in gm.
2.66	1.37	2.38	3.6	3.56	1.75	3.04	7.0
2.71	1.33	2.35	3.4	3.57	1.83	2.96	8.2
2.79	1.49	2.40	3.9	3.57	1.92	3.06	7.3
2.93	1.38	2.48	4.3	3.58	1.81	3.06	7.6
2.99	1.51	2.59	4.5	3.59	1.89	2.99	7.3
3.01	1.48	2.62	5.1	3.60	1.81	3.07	8.6
3.02	1.51	2.58	4.2	3.62	1.81	3.12	8.6
3.05	1.53	2.62	5.5	3.63	1.84	3.15	8.5
3.06	1.58	2.59	4.4	3.63	1.85	2.99	7.2
3.07	1.54	2.52	4.3	3.65	1.86	3.05	9.1
3.11	1.52	2.70	5.3	3.66	1.82	3.12	7.9
3.13	1.55	2.73	5.5	3.66	1.86	3.16	8.0
3.14	1.61	2.75	6.1	3.67	1.90	3.24	8.6
3.16	1.63	2.73	5.8	3.69	1.82	3.16	7.8
3.22	1.70	2.87	6.3	3.70	1.80	3.15	8.6
3.23	1.62	2.80	4.9	3.71	1.88	3.22	9.3
3.23	1.56	2.73	5.3	3.72	1.95	3.22	10.4
3.23	1.71	2.78	6.0	3.73	1.86	3.21	6.7
3.24	1.64	2.80	5.7	3.74	1.95	3.24	8.4
3.25	1.60	2.81	5.9	3.75	1.84	3.24	8.5
3.25	1.68	2.82	6.3	3.78	1.94	3.24	9.9
3.26	1.58	2.79	6.1	3.82	1.90	3.25	8.5
3.26	1.62	2.85	6.4	3.82	1.93	3.28	9.9
3.27	1.63	2.73	5.6	3.83	1.90	3.30	7.9
3.29	1.66	2.78	6.0	3.83	1.93	3.26	8.4
3.38	1.66	2.77	4.8	3.85	1.81	3.28	8.1
3.38	1.67	2.92	6.0	3.85	2.00	3.32	9.2
3.41	1.84	3.00	7.8	3.86	2.10	3.30	9.8
3.42	1.65	2.89	5.5	3.87	1.88	3.22	7.9
3.42	1.67	2.81	5.6	3.87	1.99	3.31	9.9
3.42	1.75	2.89	6.3	3.87	1.95	3.29	10.4
3.45	1.72	2.92	5.9	3.87	1.96	3.29	8.1
3.46	1.82	3.06	8.3	3.88	1.96	3.33	10.1
3.50	1.70	3.00	6.3	3.88	1.98	3.36	9.4
3.50	1.70	3.00	6.5	3.88	2.00	3.34	9.4
3.50	1.74	3.05	6.4	3.89	2.00	3.20	8.7
3.51	1.71	3.06	7.9	3.90	1.97	3.29	8.8
3.51	1.85	3.03	7.2	3.90	1.94	3.39	10.3
3.52	1.70	2.99	5.7	3.90	2.00	3.35	8.2
3.53	1.75	3.05	6.9	3.90	2.00	3.27	9.6
3.54	1.80	3.00	8.5	3.91	2.00	3.40	9.0
3.54	1.80	3.06	7.8	3.92	1.90	3.39	11.2
3.55	1.60	2.95	7.2	3.93	1.95	3.30	8.0

3.93	1.98	3.31	9.3	4.21	2.10	3.43	11.6
3.94	2.00	3.37	8.0	4.24	2.19	3.58	10.5
3.96	1.95	3.33	9.0	4.27	2.06	3.48	10.6
3.96	2.01	3.40	8.0	4.27	2.14	3.50	10.3
3.96	2.02	3.35	9.2	4.27	2.19	3.62	11.5
3.97	2.02	3.34	10.6	4.28	2.09	3.62	12.7
3.98	2.00	3.37	10.3	4.28	2.23	3.57	9.8
3.99	1.93	3.36	10.4	4.30	2.13	3.55	11.4
3.99	2.01	3.45	10.3	4.31	2.20	3.55	12.1
4.00	2.05	3.31	11.5	4.33	2.11	3.59	11.2
4.00	1.96	3.29	9.0	4.34	2.01	3.52	9.0
4.00	2.00	3.46	10.3	4.36	2.11	3.57	12.6
4.03	2.07	3.40	8.6	4.38	2.27	3.70	13.2
4.04	1.99	3.34	10.2	4.41	2.29	3.86	13.9
4.05	2.02	3.23	8.1	4.42	2.20	3.65	11.7
4.08	2.05	3.50	13.0	4.46	2.23	3.84	14.5
4.08	2.09	3.44	12.9	4.46	2.29	3.73	11.5
4.08	2.09	3.43	10.9	4.52	2.09	3.59	13.6
4.03	2.07	3.50	10.6	4.52	2.20	3.82	12.9
4.10	2.03	3.51	10.1	4.53	2.25	3.75	12.7
4.12	2.04	3.48	10.5	4.62	2.31	3.67	13.5
4.13	2.05	3.42	11.6	4.64	2.20	3.83	14.1
4.14	2.15	3.51	10.4	4.65	2.39	3.86	15.1
4.16	2.10	3.50	11.8	4.71	2.27	3.94	15.3
4.17	2.08	3.54	12.7	4.86	2.43	3.93	14.3
4.19	2.23	3.56	13.2	5.00	2.48	4.11	17.9
4.20	2.02	3.53	11.6	5.08	2.61	4.15	20.7
4.20	2.08	3.51	10.3				

TABLE 11. Locality I: Ōzi-Mura, Yamaguti-Ken.
Collected on July 11, 1932.

Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Length in cm.	Depth in cm.	Height in cm.	Weight in gm.
2.36	1.20	2.05	2.1	3.27	1.72	2.79	5.4
2.50	1.35	2.21	2.6	3.27	1.75	2.78	5.3
2.50	1.39	2.19	2.9	3.31	1.71	2.84	5.0
2.71	1.38	2.38	3.3	3.33	1.65	2.86	5.8
2.71	1.47	2.35	3.4	3.36	1.83	2.89	6.2
2.77	1.44	2.35	3.4	3.40	1.79	2.90	6.0
2.80	1.41	2.44	3.6	3.45	1.86	2.93	6.3
2.91	1.63	2.51	4.4	3.46	1.71	2.92	5.5
2.94	1.50	2.52	3.5	3.47	1.82	2.99	6.6
2.95	1.55	2.56	4.5	3.48	1.78	2.89	6.4
3.00	1.50	2.55	4.0	3.48	1.81	3.01	5.1
3.00	1.58	2.63	4.7	3.49	1.78	2.98	6.5
3.00	1.54	2.63	4.3	3.52	1.82	3.04	6.8
3.02	1.59	2.60	4.1	3.52	1.92	3.08	7.6
3.04	1.64	2.70	5.0	3.53	1.79	3.12	6.7
3.07	1.68	2.67	5.1	3.58	1.96	3.11	7.5
3.09	1.61	2.65	4.5	3.61	1.91	2.95	6.7
3.09	1.62	2.66	4.6	3.85	1.96	3.21	6.8
3.10	1.65	2.75	4.9	3.94	2.11	3.32	9.7
3.16	1.70	2.69	5.4	4.08	2.12	3.36	9.0
3.17	1.66	2.69	5.1	4.16	2.18	3.48	9.9
3.19	1.78	2.80	6.2	4.37	2.10	3.57	8.8
3.20	1.61	2.73	5.4	4.42	2.30	3.72	11.5
3.25	1.85	2.79	6.0	4.43	2.34	3.68	10.5

4.53	2.24	3.87	12.0	4.93	2.56	4.16	15.0
4.57	2.25	3.83	11.7	5.06	2.67	4.20	15.5
4.60	2.27	3.64	10.2	5.15	2.51	4.19	15.3
4.64	2.43	3.92	13.1	5.20	2.73	4.53	18.9
4.68	2.46	3.82	14.0	5.37	2.85	4.54	20.4
4.70	2.36	3.79	12.3	5.44	2.76	4.40	19.2
4.71	2.49	3.90	13.4	5.81	2.91	4.74	22.4
4.72	2.47	3.97	13.3	5.90	2.93	4.79	26.9
4.74	2.55	3.85	14.1	5.93	3.06	4.87	24.4
4.79	2.47	3.91	13.7	6.00	3.21	4.89	28.5
4.80	2.25	3.88	12.4	6.26	3.12	5.14	25.4
4.81	2.44	3.92	12.1	6.43	3.37	5.29	30.9
4.85	2.48	4.01	14.2	6.45	3.38	5.27	36.5
4.87	2.41	3.98	13.5	6.51	3.37	5.12	31.8
4.87	2.47	4.07	12.8	6.55	3.42	5.46	37.8
4.92	2.50	3.98	14.5	7.70	4.13	6.27	62.1
4.92	2.49	3.78	13.1	7.73	3.92	6.32	48.5

TABLE 12. Locality J: Kawagoe-Mura, Miye-Ken.
Collected on Sept. 29, 1932.

Length in cm.	Depth in cm	Height in cm.	Weight in gm	Length in cm	Depth in cm	Height in cm	Weight in gm.
2.13	1.04	1.81	1.6	2.82	1.49	2.34	3.3
2.22	1.08	1.86	1.9	2.87	1.45	2.34	3.7
2.33	1.17	1.91	1.9	2.89	1.43	2.39	3.5
2.37	1.21	1.98	2.2	2.91	1.43	2.40	2.9
2.38	1.15	2.00	2.2	2.96	1.51	2.37	3.3
2.40	1.25	2.00	2.2	2.97	1.55	2.40	3.6
2.42	1.21	2.01	2.3	2.98	1.44	2.43	3.4
2.43	1.20	1.99	2.2	3.01	1.46	2.44	3.6
2.45	1.23	2.16	2.7	3.01	1.49	2.42	3.5
2.45	1.24	2.10	2.4	3.03	1.60	2.55	4.2
2.47	1.20	2.10	2.7	3.05	1.54	2.60	4.3
2.47	1.22	2.04	2.3	3.09	1.52	2.55	4.6
2.48	1.23	2.07	2.5	3.10	1.60	2.55	4.3
2.48	1.25	2.05	2.7	3.11	1.56	2.59	4.0
2.50	1.25	2.08	2.5	3.11	1.61	2.51	4.3
2.53	1.31	2.19	2.9	3.12	1.54	2.53	4.1
2.56	1.31	2.21	2.9	3.13	1.49	2.53	3.7
2.58	1.31	2.16	2.7	3.15	1.56	2.60	4.4
2.61	1.31	2.20	2.8	3.16	1.62	2.61	4.3
2.63	1.32	2.19	2.9	3.17	1.61	2.56	4.1
2.65	1.37	2.27	3.4	3.17	1.70	2.59	4.7
2.65	1.29	2.20	3.0	3.18	1.62	2.62	4.1
2.66	1.28	2.21	2.3	3.21	1.63	2.57	4.4
2.67	1.34	2.23	2.6	3.23	1.56	2.62	4.8
2.71	1.32	2.30	3.1	3.23	1.66	2.60	4.3
2.71	1.34	2.27	3.0	3.24	1.58	2.73	4.7
2.75	1.47	2.32	3.3	3.24	1.59	2.58	4.2
2.76	1.36	2.29	3.0	3.24	1.61	2.63	4.8
2.76	1.46	2.31	3.4	3.29	1.67	2.63	4.8
2.77	1.40	2.28	3.4	3.30	1.61	2.71	4.3
2.78	1.36	2.33	3.3	3.30	1.59	2.66	5.3
2.80	1.39	2.28	2.9	3.30	1.71	2.71	4.9
2.80	1.41	2.33	3.2	3.30	1.71	2.76	4.4
2.81	1.44	2.21	2.4	3.31	1.69	2.76	5.1
2.82	1.41	2.32	2.9	3.32	1.60	2.60	4.4

Length in cm	Depth in cm	Height in cm.	Weight in gm.	Length in cm	Depth in cm	Height in cm	Weight in gm
3.33	1.72	2.77	4.8	3.81	1.94	3.11	6.2
3.34	1.76	2.70	5.0	3.81	1.94	3.09	6.8
3.35	1.63	2.68	4.4	3.82	1.90	3.02	7.2
3.35	1.70	2.71	4.1	3.83	1.93	3.00	6.3
3.38	1.67	2.72	4.6	3.83	1.79	3.05	6.4
3.38	1.71	2.80	5.5	3.83	2.03	3.18	7.9
3.40	1.73	2.76	5.1	3.84	1.88	3.10	6.5
3.40	1.75	2.84	5.5	3.84	1.90	3.10	6.4
3.41	1.67	2.75	4.7	3.86	1.91	3.12	6.9
3.42	1.75	2.77	4.8	3.86	1.90	3.08	6.4
3.42	1.76	2.76	5.5	3.87	1.95	3.00	6.0
3.46	1.78	2.88	5.2	3.87	1.99	3.10	7.5
3.47	1.76	2.78	4.7	3.88	1.94	3.05	6.9
3.48	1.73	2.85	5.6	3.88	1.93	3.10	7.2
3.48	1.76	2.86	5.2	3.90	1.93	3.13	6.9
3.48	1.79	2.86	5.3	3.92	1.97	3.15	8.0
3.49	1.70	2.80	4.5	3.94	1.96	3.09	7.0
3.49	1.85	2.81	5.4	3.94	1.93	3.14	6.7
3.50	1.82	2.79	6.1	3.95	2.01	3.17	7.4
3.51	1.71	2.89	5.4	3.98	1.99	3.20	7.1
3.52	1.84	2.84	5.1	3.99	2.00	3.08	6.4
3.52	1.82	2.84	5.2	3.99	2.06	3.18	7.0
3.53	1.83	2.85	5.3	4.01	2.02	3.19	7.7
3.54	1.71	2.84	5.3	4.06	2.10	3.27	7.4
3.55	1.77	2.90	6.3	4.09	2.13	3.19	8.3
3.56	1.87	2.85	5.9	4.11	2.01	3.27	6.5
3.57	1.74	2.83	5.7	4.11	2.08	3.28	8.4
3.57	1.78	2.86	6.1	4.12	2.02	3.15	7.0
3.57	1.80	2.87	5.4	4.16	2.06	3.30	8.1
3.58	1.85	2.90	5.5	4.19	2.14	3.48	9.9
3.58	1.78	2.90	5.8	4.19	2.27	3.45	9.1
3.59	1.71	2.87	5.5	4.38	2.20	3.53	9.8
3.59	1.75	2.88	5.2	4.39	2.19	3.53	8.8
3.60	1.85	2.99	5.5	4.49	2.19	3.62	9.8
3.60	1.76	2.91	5.2	4.50	2.25	3.47	9.9
3.61	1.81	2.90	5.2	4.53	2.28	3.59	10.2
3.61	1.83	2.85	6.3	4.59	2.27	3.60	10.1
3.62	1.85	2.93	6.0	4.59	2.41	3.66	11.2
3.65	1.76	2.93	6.1	4.68	2.36	3.70	11.5
3.66	1.81	3.01	5.5	4.70	2.44	3.70	11.2
3.67	1.85	2.97	5.9	4.73	2.31	3.71	10.8
3.67	1.87	2.98	6.1	4.76	2.45	3.78	12.0
3.69	1.80	3.06	6.3	4.85	2.45	3.84	13.8
3.70	1.94	2.94	5.9	4.89	2.36	3.90	11.4
3.70	1.82	2.98	6.0	4.89	2.45	3.85	11.6
3.71	1.83	2.92	5.7	4.90	2.41	3.85	12.8
3.71	1.91	3.01	6.7	4.96	2.37	3.95	12.2
3.71	1.87	2.95	6.2	5.01	2.49	3.90	14.1
3.73	1.79	2.95	6.0	5.13	2.59	4.01	13.2
3.75	1.85	2.94	5.6	5.17	2.60	4.07	16.2
3.76	1.84	3.03	5.7	5.23	2.54	4.06	13.9
3.77	1.96	3.04	6.5	5.37	2.68	4.10	17.0
3.78	1.94	3.07	6.1	5.54	2.79	4.36	18.7
3.79	1.89	2.99	6.1	5.57	2.76	4.31	19.6
3.80	1.86	2.94	6.2	5.59	2.83	4.30	19.9
3.81	1.81	3.07	5.9				

TABLE 13. Locality K: Kankawa, Tiba-Ken.
Collected on Sept. 30, 1932.

Length in cm	Depth in cm	Height in cm	Weight in gm	Length in cm	Depth in cm	Height in cm	Weight in gm.
1.48	0.70	1.22	0.5	2.52	1.22	2.09	2.0
1.60	0.72	1.34	0.6	2.52	1.21	2.07	2.0
1.78	0.80	1.50	0.8	2.53	1.16	2.09	2.1
1.82	0.83	1.53	0.8	2.54	1.18	2.11	2.0
1.88	0.86	1.52	0.9	2.54	1.20	2.12	2.2
1.93	0.87	1.63	1.0	2.55	1.14	2.12	2.0
1.96	0.91	1.67	1.1	2.55	1.19	2.05	2.0
2.00	0.94	1.67	1.1	2.56	1.17	2.17	2.1
2.15	1.00	1.82	1.3	2.57	1.22	2.08	2.1
2.16	1.00	1.79	1.4	2.57	1.13	2.09	2.1
2.19	0.95	1.78	1.2	2.58	1.23	2.14	2.2
2.20	0.96	1.79	1.2	2.58	1.27	2.14	2.3
2.20	1.01	1.83	1.3	2.60	1.17	2.14	2.0
2.20	1.01	1.85	1.3	2.62	1.25	2.13	1.9
2.21	1.06	1.88	1.4	2.63	1.21	2.14	2.2
2.21	1.05	1.87	1.6	2.64	1.20	2.12	2.0
2.24	1.05	1.79	1.3	2.64	1.23	2.11	2.2
2.25	1.01	1.84	1.4	2.67	1.24	2.15	2.0
2.25	1.06	1.91	1.6	2.68	1.23	2.17	2.4
2.26	1.07	2.00	1.6	2.68	1.20	2.20	2.0
2.26	1.03	1.97	1.5	2.68	1.23	2.11	2.0
2.28	1.02	1.93	1.2	2.70	1.24	2.22	2.0
2.29	1.05	1.93	1.4	2.70	1.23	2.14	2.2
2.29	1.08	1.85	1.6	2.73	1.35	2.22	2.6
2.30	1.07	1.91	1.5	2.74	1.37	2.28	2.8
2.32	1.02	1.93	1.6	2.76	1.33	2.20	2.6
2.34	1.10	1.96	1.7	2.76	1.29	2.30	2.6
2.34	1.13	1.96	1.8	2.78	1.21	2.21	2.3
2.35	1.09	1.95	1.7	2.79	1.30	2.25	2.9
2.35	1.07	1.93	1.5	2.80	1.27	2.25	2.5
2.35	1.09	1.96	1.5	2.81	1.29	2.25	2.5
2.36	1.10	1.93	1.7	2.81	1.33	2.26	2.6
2.36	1.14	2.04	1.9	2.84	1.30	2.31	2.7
2.36	1.10	1.97	1.5	2.85	1.27	2.29	2.7
2.36	1.14	1.95	1.5	2.85	1.35	2.11	3.2
2.38	1.14	1.91	1.6	2.88	1.32	2.30	2.8
2.39	1.12	2.00	1.6	2.83	1.33	2.31	2.9
2.40	1.10	1.95	1.9	2.90	1.37	2.37	3.0
2.41	1.14	1.99	1.9	2.91	1.42	2.42	3.2
2.41	1.07	1.93	1.6	2.92	1.38	2.36	2.8
2.43	1.13	1.97	1.7	2.96	1.37	2.14	3.1
2.45	1.12	2.01	1.9	2.96	1.38	2.35	3.4
2.46	1.18	2.03	1.8	2.97	1.42	2.38	2.8
2.47	1.12	2.03	1.7	3.00	1.33	2.25	2.5
2.48	1.12	1.96	1.7	3.00	1.45	2.45	3.6
2.49	1.20	2.04	1.9	3.00	1.38	2.40	2.7
2.49	1.14	1.99	1.8	3.01	1.44	2.49	3.4
2.50	1.13	2.03	1.7	3.03	1.39	2.45	3.2
2.50	1.15	2.10	1.9	3.04	1.40	2.45	3.4
2.51	1.15	2.10	1.9	3.04	1.41	2.46	3.4
2.51	1.21	2.06	2.0	3.05	1.36	2.48	3.0
2.51	1.20	2.01	1.9	3.05	1.46	2.44	3.0
2.51	1.25	2.07	2.1	3.06	1.46	2.53	3.2
2.51	1.21	2.11	2.1	3.07	1.54	2.58	3.7
2.52	1.13	2.10	1.8	3.09	1.43	2.59	3.4

Length in cm.	Depth in cm.	Height in cm	Weight in gm.	Length in cm.	Depth in cm.	Height in cm	Weight in gm
3.10	1.44	2.52	3.3	3.46	1.57	2.67	4.3
3.11	1.47	2.46	3.6	3.46	1.57	2.79	4.4
3.13	1.50	2.50	3.2	3.47	1.69	2.82	4.9
3.14	1.44	2.51	3.3	3.47	1.66	2.79	4.7
3.16	1.50	2.50	3.6	3.47	1.74	2.89	5.2
3.17	1.50	2.56	3.7	3.48	1.80	2.89	4.3
3.18	1.48	2.51	3.2	3.48	1.65	2.82	4.7
3.18	1.51	2.63	3.8	3.48	1.78	2.77	4.9
3.18	1.40	2.59	3.3	3.48	1.65	2.77	5.3
3.18	1.59	2.56	3.8	3.49	1.60	2.75	4.9
3.19	1.51	2.57	3.8	3.49	1.60	2.79	4.5
3.19	1.48	2.58	3.4	3.49	1.64	2.81	4.8
3.19	1.49	2.58	3.4	3.49	1.57	2.78	4.4
3.20	1.48	2.59	3.7	3.50	1.67	2.76	4.4
3.21	1.50	2.59	4.1	3.50	1.69	2.82	5.1
3.21	1.47	2.58	3.4	3.50	1.57	2.81	4.2
3.23	1.49	2.57	3.4	3.50	1.59	2.83	5.0
3.24	1.51	2.67	3.7	3.50	1.65	2.85	4.8
3.25	1.55	2.64	4.3	3.50	1.66	2.83	4.9
3.25	1.50	2.59	3.7	3.51	1.61	2.80	4.8
3.25	1.56	2.55	3.8	3.51	1.65	2.80	4.4
3.25	1.51	2.58	3.7	3.52	1.68	2.80	4.7
3.25	1.58	2.65	4.2	3.53	1.60	2.86	4.9
3.27	1.50	2.68	3.7	3.53	1.66	2.82	4.7
3.28	1.50	2.69	4.1	3.53	1.65	2.89	4.6
3.28	1.55	2.59	4.0	3.54	1.66	2.82	4.5
3.29	1.56	2.66	4.3	3.54	1.63	2.84	4.6
3.29	1.56	2.70	4.5	3.54	1.68	2.89	4.8
3.30	1.48	2.59	3.6	3.55	1.70	2.88	4.7
3.30	1.55	2.60	3.8	3.55	1.74	2.83	5.3
3.31	1.60	2.66	4.1	3.58	1.78	2.86	5.3
3.32	1.65	2.70	4.5	3.58	1.63	2.90	4.7
3.32	1.57	2.73	4.3	3.59	1.65	2.86	4.7
3.33	1.56	2.66	4.1	3.59	1.72	2.81	5.1
3.33	1.59	2.66	4.3	3.59	1.76	2.88	5.6
3.34	1.51	2.66	3.7	3.60	1.67	2.75	5.1
3.34	1.62	2.72	4.4	3.60	1.73	2.88	5.3
3.35	1.55	2.70	3.9	3.60	1.69	2.82	4.8
3.35	1.52	2.72	3.8	3.61	1.68	2.95	5.4
3.35	1.49	2.70	3.8	3.63	1.77	2.83	4.9
3.35	1.55	2.63	4.0	3.63	1.71	2.88	5.2
3.35	1.54	2.70	4.0	3.63	1.80	2.84	5.6
3.36	1.61	2.68	4.4	3.64	1.82	2.99	5.7
3.37	1.56	2.75	4.2	3.65	1.71	2.91	5.3
3.37	1.60	2.67	4.4	3.65	1.71	2.99	5.2
3.38	1.61	2.72	4.8	3.65	1.80	2.86	5.5
3.38	1.54	2.65	3.8	3.65	1.75	3.03	5.7
3.38	1.55	2.69	4.1	3.66	1.63	2.89	5.0
3.38	1.53	2.75	4.2	3.66	1.73	2.89	5.2
3.39	1.59	2.75	4.5	3.67	1.82	2.99	6.2
3.40	1.63	2.81	4.7	3.68	1.68	2.97	5.0
3.40	1.56	2.77	4.2	3.68	1.67	2.91	5.1
3.41	1.65	2.83	4.5	3.69	1.70	2.87	4.4
3.41	1.66	2.79	4.6	3.69	1.80	3.01	5.6
3.42	1.63	2.71	4.1	3.70	1.80	3.00	5.7
3.42	1.56	2.78	4.3	3.70	1.83	3.03	5.7
3.42	1.67	2.79	4.7	3.71	1.80	2.94	5.6
3.44	1.55	2.75	4.7	3.72	1.77	2.93	5.0
3.45	1.68	2.68	4.6	3.72	1.73	2.95	5.0

3.72	1.81	3.31	5.8	3.91	1.75	3.09	6.7
3.73	1.68	2.95	5.5	3.91	1.80	3.14	6.1
3.73	1.80	2.96	5.7	3.92	1.85	3.10	6.4
3.74	1.84	3.09	6.6	3.93	1.93	3.09	6.9
3.74	1.79	2.99	5.5	3.93	1.85	3.04	6.1
3.75	1.78	2.87	5.5	3.95	1.90	3.12	6.7
3.75	1.75	2.88	5.5	4.02	2.01	3.27	7.6
3.76	1.72	2.99	5.6	4.03	2.01	3.21	7.3
3.76	1.74	3.01	5.5	4.04	1.82	3.17	6.1
3.78	1.79	3.04	5.8	4.12	1.93	3.23	6.5
3.79	1.75	3.00	5.4	4.16	1.99	3.33	8.1
3.80	1.82	2.97	5.6	4.19	1.99	3.32	8.0
3.80	1.80	3.06	5.8	4.41	2.08	3.57	8.8
3.81	1.85	3.00	5.5	4.47	2.04	3.41	9.1
3.81	1.82	3.04	5.9	4.52	2.15	3.59	8.7
3.82	1.74	3.06	5.4	4.76	2.24	3.60	10.2
3.83	1.70	2.99	5.5	4.84	2.34	3.80	10.3
3.85	1.76	3.01	6.0	4.86	2.21	3.72	10.4
3.86	1.77	3.04	6.1	5.05	2.40	3.92	12.5
3.86	1.80	3.08	6.5	5.32	2.47	4.10	14.9
3.90	1.88	3.13	6.6	5.47	2.57	4.15	15.3
3.90	1.90	3.17	6.7	5.56	2.53	4.04	15.9
3.90	1.91	3.12	5.9	5.95	2.64	4.51	19.1

TABLE 14. Locality L: Hattori-Mura, Tottori-Ken.
Collected on Oct. 3, 1932.

Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Length in cm.	Depth in cm.	Height in cm.	Weight in gm.
5.80	2.82	4.60	30.9	7.27	3.42	5.72	63.1
5.81	2.53	4.45	28.3	7.28	3.49	5.73	63.3
6.36	2.96	4.84	38.7	7.29	3.45	5.70	60.2
6.45	3.08	4.98	39.0	7.29	3.33	5.54	59.2
6.50	3.01	5.13	43.2	7.32	3.10	5.48	50.3
6.67	3.01	5.20	48.4	7.33	3.52	5.73	63.5
6.84	3.04	5.23	41.8	7.37	3.39	5.62	60.1
6.89	3.27	5.34	48.5	7.40	3.33	5.76	63.4
6.93	3.10	5.38	52.8	7.43	3.41	5.93	63.2
7.07	3.06	5.34	47.8	7.41	3.43	5.69	62.3
7.08	3.30	5.50	53.2	7.47	3.51	5.75	65.7
7.09	3.18	5.29	51.3	7.52	3.33	5.71	61.5
7.10	3.24	5.37	52.0	7.55	3.46	5.75	66.8
7.22	3.48	5.65	60.9	7.67	3.60	5.81	71.1
7.24	3.24	5.58	59.9	7.69	3.48	5.70	66.3

TABLE 15. Locality M: Matukawa-Ura, Hukusima-Ken.
Collected on Sept. 2, 1933.

Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Length in cm.	Depth in cm.	Height in cm.	Weight in gm.
0.52	0.24	0.46		0.78	0.35	0.69	
0.70	0.33	0.61		0.79	0.38	0.68	
0.74	0.35	0.65		0.80	0.37	0.69	
0.78	0.36	0.68		0.80	0.38	0.72	

Length in cm	Depth in cm.	Height in cm	Weight in gm	Length in cm.	Depth in cm	Height in cm	Weight in gm.
0.82	0.38	0.70		1.33	0.61	1.13	
0.84	0.38	0.71		1.34	0.58	1.15	
0.84	0.39	0.70		1.34	0.62	1.12	
0.84	0.40	0.72		1.34	0.62	1.17	
0.85	0.39	0.72		1.35	0.60	1.16	
0.85	0.40	0.75		1.35	0.61	1.14	
0.86	0.41	0.75		1.35	0.61	1.16	
0.86	0.41	0.75		1.37	0.65	1.18	
0.88	0.40	0.73		1.38	0.61	1.15	
0.90	0.41	0.77		1.40	0.64	1.20	
0.90	0.43	0.78		1.42	0.62	1.19	
0.91	0.40	0.79		1.45	0.67	1.23	
0.91	0.41	0.79		1.46	0.72	1.27	
0.91	0.43	0.77		1.48	0.67	1.26	
0.92	0.43	0.78		1.50	0.71	1.27	
0.92	0.44	0.81		1.51	0.69	1.29	
0.93	0.44	0.80		1.53	0.72	1.28	
0.93	0.45	0.82		1.55	0.70	1.32	
0.94	0.43	0.80		1.55	0.70	1.35	
0.98	0.42	0.81		1.56	0.72	1.31	
0.99	0.43	0.81		1.57	0.73	1.36	0.8
0.99	0.44	0.86		1.70	0.87	1.51	1.1
1.00	0.45	0.84		1.71	0.78	1.42	0.9
1.00	0.46	0.87		1.75	0.88	1.56	1.1
1.01	0.45	0.86		1.79	0.86	1.57	1.1
1.05	0.49	0.91		1.86	0.86	1.59	1.2
1.07	0.47	0.95		1.88	0.88	1.63	1.3
1.08	0.48	0.92		1.88	0.91	1.68	1.3
1.08	0.49	0.91		1.93	0.91	1.66	1.5
1.08	0.49	0.94		1.94	0.86	1.67	1.3
1.10	0.50	0.94		1.97	0.89	1.71	1.4
1.10	0.51	0.95		1.97	0.93	1.69	1.4
1.10	0.51	0.95		2.00	0.92	1.71	1.5
1.11	0.52	0.97		2.04	0.98	1.77	1.8
1.12	0.52	0.93		2.09	0.96	1.81	1.7
1.13	0.51	0.95		2.11	1.03	1.80	1.9
1.15	0.51	1.00		2.12	1.04	1.83	2.0
1.17	0.54	1.01		2.14	1.00	1.85	1.9
1.18	0.52	1.00		2.14	1.02	1.82	1.8
1.18	0.53	1.00		2.14	1.03	1.84	1.9
1.19	0.52	1.02		2.15	1.04	1.88	1.9
1.19	0.53	1.01		2.20	1.03	1.86	1.8
1.20	0.58	1.05		2.20	1.06	1.90	2.1
1.21	0.53	1.02		2.22	1.01	1.93	2.0
1.21	0.59	1.07		2.30	1.11	2.00	2.5
1.22	0.58	1.05		2.31	1.12	2.02	2.4
1.25	0.57	1.06		2.31	1.17	2.00	2.5
1.25	0.59	1.06		2.31	1.17	2.03	2.6
1.26	0.59	1.10		2.39	1.06	2.00	2.3
1.28	0.60	1.08		2.39	1.16	2.05	2.6
1.29	0.59	1.11		2.40	1.15	2.13	2.6
1.30	0.60	1.10		2.40	1.21	2.10	2.9
1.30	0.60	1.12		2.42	1.20	2.11	3.0
1.30	0.59	1.13		2.45	1.12	2.10	2.6
1.31	0.60	1.10		2.47	1.13	2.13	2.7
1.32	0.59	1.12		2.50	1.20	2.15	2.8
1.33	0.59	1.15		2.53	1.21	2.14	2.9
1.33	0.60	1.11		2.59	1.21	2.19	2.9
1.33	0.61	1.13		2.60	1.22	2.20	2.8

2.61	1.22	2.24	3.2	3.10	1.41	2.65	5.0
2.61	1.23	2.26	3.1	3.10	1.50	2.67	5.2
2.61	1.24	2.19	3.0	3.15	1.49	2.67	5.0
2.62	1.32	2.32	3.6	3.17	1.57	2.66	5.8
2.64	1.22	2.28	3.4	3.19	1.53	2.79	5.5
2.65	1.18	2.20	3.0	3.21	1.54	2.70	5.3
2.66	1.32	2.38	3.9	3.23	1.61	2.71	5.7
2.67	1.28	2.30	3.7	3.24	1.61	2.78	6.2
2.68	1.28	2.29	3.4	3.25	1.50	2.74	5.9
2.69	1.31	2.26	3.3	3.26	1.56	2.79	5.8
2.70	1.30	2.30	3.8	3.27	1.62	2.80	6.3
2.71	1.30	2.31	3.8	3.29	1.61	2.87	6.9
2.71	1.31	2.37	3.8	3.31	1.57	2.85	5.9
2.71	1.32	2.32	3.8	3.34	1.67	2.82	6.2
2.71	1.36	2.30	3.9	3.35	1.50	2.76	4.9
2.75	1.31	2.30	3.6	3.36	1.72	2.88	6.8
2.75	1.32	2.36	3.8	3.37	1.67	2.82	6.0
2.78	1.29	2.39	3.8	3.40	1.61	2.85	6.2
2.78	1.33	2.37	4.2	3.40	1.68	2.80	6.3
2.80	1.31	2.35	3.9	3.40	1.70	2.83	6.9
2.80	1.37	2.42	4.2	3.43	1.61	2.90	6.0
2.81	1.29	2.42	4.0	3.53	1.73	2.93	7.3
2.81	1.44	2.38	4.3	3.54	1.72	2.95	6.9
2.85	1.38	2.43	4.3	3.54	1.79	3.00	7.7
2.92	1.42	2.52	4.6	3.55	1.70	3.06	7.1
2.92	1.43	2.44	4.4	3.55	1.81	3.01	7.5
2.99	1.41	2.54	4.6	3.71	1.79	3.07	7.8
3.02	1.47	2.53	4.7	3.73	1.81	3.15	8.2
3.05	1.59	2.58	4.9	3.79	1.92	3.16	9.2
3.05	1.48	2.63	4.7	3.87	1.85	3.19	8.4
3.06	1.50	2.60	5.2				

TABLE 16. Locality N: Watanoha-Mati, Miyagi-Ken.
Collected on Sept. 19, 1933.

Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Length in cm.	Depth in cm.	Height in cm.	Weight in gm.
1.17	0.45	0.99	0.2	3.45	1.55	2.75	6.5
1.40	0.53	1.13	0.4	3.48	1.54	2.70	6.9
1.41	0.51	1.13	0.4	3.62	1.59	2.82	7.3
1.41	0.51	1.16	0.4	3.65	1.57	2.82	7.2
1.57	0.58	1.31	0.5	3.67	1.72	2.91	7.7
1.60	0.58	1.29	0.5	3.71	1.63	2.79	7.2
1.61	0.60	1.28	0.5	3.75	1.71	2.98	8.3
2.00	0.78	1.61	1.1	3.78	1.80	3.04	8.9
2.05	0.81	1.70	1.3	3.80	1.77	3.00	9.0
2.48	1.07	2.08	2.4	3.83	1.70	2.97	9.0
2.66	1.12	2.11	2.7	3.86	1.77	3.03	8.2
2.72	1.18	2.27	3.1	3.90	1.75	3.05	10.0
2.77	1.22	2.21	3.6	3.91	1.85	3.08	9.9
2.84	1.27	2.28	3.8	3.98	1.72	3.13	9.4
2.86	1.20	2.30	3.4	3.98	1.84	3.20	10.3
2.95	1.28	2.31	3.8	4.00	1.81	3.24	9.9
3.05	1.33	2.43	4.4	4.11	2.01	3.30	12.0
3.10	1.42	2.52	4.9	4.14	1.90	3.23	10.9
3.20	1.38	2.56	5.0	4.22	2.00	3.32	12.0
3.34	1.46	2.58	5.8	4.27	2.02	3.33	12.6
3.40	1.50	2.70	6.2	4.28	1.89	3.29	11.2

4.31	1.95	3.38	12.7	4.72	2.14	3.72	15.5
4.34	2.00	3.31	12.6	4.75	2.08	3.65	15.0
4.35	2.04	3.34	12.8	4.87	2.13	3.72	15.5
4.37	2.02	3.43	13.3	4.95	2.38	3.88	20.5
4.39	1.91	3.46	13.3	5.19	2.41	3.95	20.8
4.42	2.06	3.41	13.2	5.20	2.42	4.09	21.1
4.42	2.11	3.58	14.1	5.27	2.37	3.92	21.4
4.55	2.06	3.51	14.0	5.41	2.47	4.29	23.9
4.56	2.10	3.60	15.2	5.42	2.50	4.24	23.5
4.57	2.04	3.49	14.4	5.44	2.50	4.05	23.3
4.58	2.17	3.67	16.9	5.60	2.46	4.20	24.7
4.66	2.20	3.72	17.7	6.15	2.87	4.80	34.3
4.71	2.22	3.67	16.4				

TABLE 17. Locality P: Kusatu-Mati, Hirosima-Ken.
Collected on Feb. 14, 1933.

Length in cm	Depth in cm	Height in cm	Weight in gm	Length in cm.	Depth in cm.	Height in cm.	Weight in gm
2.39	1.08	1.90	1.6	2.72	1.44	2.26	3.2
2.34	1.12	1.98	1.8	2.73	1.36	2.28	2.4
2.37	1.18	2.01	1.6	2.73	1.39	2.32	2.9
2.37	1.21	1.99	1.9	2.73	1.39	2.32	2.6
2.38	1.16	2.04	1.9	2.73	1.40	2.25	2.6
2.40	1.20	2.05	1.8	2.73	1.42	2.33	2.6
2.41	1.20	2.09	2.2	2.74	1.32	2.31	2.4
2.44	1.26	2.11	2.5	2.74	1.33	2.30	2.6
2.48	1.26	2.13	2.3	2.74	1.34	2.35	2.5
2.51	1.22	2.09	2.0	2.75	1.49	2.34	3.3
2.52	1.29	2.16	2.3	2.76	1.34	2.31	3.3
2.53	1.20	2.10	2.0	2.76	1.36	2.30	2.6
2.53	1.25	2.13	2.6	2.76	1.37	2.33	2.8
2.56	1.30	2.26	2.5	2.76	1.38	2.31	2.6
2.57	1.28	2.21	2.5	2.77	1.35	2.32	2.9
2.58	1.26	2.18	2.4	2.77	1.37	2.24	2.6
2.59	1.24	2.21	2.1	2.77	1.37	2.30	2.2
2.59	1.29	2.22	2.6	2.77	1.38	2.36	2.7
2.59	1.30	2.17	2.4	2.77	1.42	2.32	2.7
2.59	1.37	2.27	2.5	2.78	1.35	2.30	2.9
2.60	1.26	2.12	2.3	2.78	1.37	2.37	3.6
2.60	1.26	2.25	2.4	2.79	1.36	2.45	2.9
2.61	1.26	2.15	2.2	2.79	1.38	2.36	2.9
2.61	1.30	2.22	2.5	2.79	1.43	2.35	3.1
2.61	1.30	2.23	2.3	2.80	1.36	2.34	2.8
2.63	1.32	2.19	2.3	2.80	1.39	2.29	2.6
2.63	1.36	2.23	2.2	2.80	1.40	2.42	3.0
2.64	1.34	2.21	2.6	2.80	1.45	2.39	3.2
2.64	1.41	2.25	3.1	2.80	1.46	2.38	3.1
2.65	1.32	2.25	2.4	2.80	1.47	2.32	2.7
2.66	1.28	2.26	2.3	2.81	1.39	2.32	3.0
2.66	1.29	2.21	2.6	2.81	1.44	2.36	2.7
2.66	1.36	2.28	3.0	2.81	1.45	2.33	2.3
2.66	1.40	2.28	2.7	2.82	1.31	2.29	2.8
2.67	1.33	2.28	2.8	2.82	1.43	2.33	2.7
2.67	1.34	2.22	2.5	2.82	1.47	2.32	3.0
2.67	1.34	2.26	2.7	2.83	1.40	2.40	3.2
2.71	1.35	2.40	2.6	2.83	1.43	2.41	3.0
2.71	1.36	2.35	2.9	2.84	1.27	2.33	2.4

Length in cm.	Depth in cm	Height in cm	Weight in gm.	Length in cm	Depth in cm.	Height in cm	Weight in gm.
2.84	1.45	2.34	2.9	3.36	1.68	2.80	4.7
2.84	1.45	2.45	3.4	3.37	1.72	2.80	5.0
2.85	1.40	2.44	3.0	3.37	1.80	2.81	5.3
2.85	1.42	2.31	3.2	3.38	1.72	2.78	5.1
2.86	1.46	2.37	2.9	3.39	1.80	2.66	4.7
2.87	1.42	2.42	3.2	3.40	1.73	2.78	4.3
2.87	1.45	2.51	3.5	3.41	1.62	2.79	4.8
2.87	1.55	2.50	3.5	3.42	1.72	2.81	4.8
2.88	1.36	2.38	2.8	3.44	1.67	2.82	5.2
2.88	1.42	2.42	3.4	3.46	1.69	2.89	5.1
2.89	1.41	2.43	3.0	3.46	1.71	2.89	5.4
3.90	1.44	2.43	2.9	3.46	1.80	2.79	4.6
2.91	1.52	2.49	3.1	3.46	1.84	2.94	5.7
2.92	1.44	2.44	2.9	3.47	1.73	2.83	5.2
2.92	1.47	2.45	3.3	3.48	1.89	2.93	5.1
2.92	1.50	2.47	3.3	3.49	1.76	2.77	5.2
2.93	1.47	2.42	3.7	3.52	1.79	2.90	5.9
2.94	1.40	2.40	3.0	3.53	1.80	2.87	5.9
2.95	1.42	2.48	3.5	3.53	1.80	2.91	5.7
2.95	1.49	2.46	3.9	3.56	1.85	2.85	5.8
2.95	1.52	2.48	3.1	3.57	1.89	2.96	5.7
2.96	1.48	2.44	3.2	3.57	1.90	3.00	6.4
2.96	1.50	2.42	3.2	3.59	1.91	2.86	5.8
2.98	1.55	2.50	3.6	3.60	1.77	2.89	5.0
2.99	1.49	2.52	3.4	3.62	1.81	2.97	5.6
3.00	1.45	2.40	3.6	3.71	1.91	3.00	5.7
3.01	1.49	2.43	3.7	3.75	1.87	3.11	6.6
3.01	1.58	2.52	4.1	3.80	1.94	3.06	5.6
3.04	1.57	2.65	3.6	3.81	1.89	3.07	6.9
3.05	1.54	2.51	3.7	3.83	1.99	3.15	7.0
3.05	1.61	2.58	4.7	3.88	1.98	3.03	7.3
3.06	1.63	2.55	3.8	3.92	2.06	3.16	7.7
3.07	1.51	2.49	3.4	3.96	2.05	3.29	7.9
3.07	1.54	2.58	4.0	4.03	2.05	3.25	8.0
3.10	1.51	2.57	3.9	4.05	1.99	3.34	7.1
3.10	1.55	2.50	4.3	4.06	2.10	3.32	7.5
3.12	1.53	2.65	4.1	4.12	2.12	3.31	8.4
3.12	1.54	2.58	3.2	4.22	1.99	3.46	8.2
3.12	1.57	2.57	4.1	4.22	2.18	3.48	8.8
3.13	1.57	2.53	4.5	4.23	2.15	3.60	9.7
3.15	1.61	2.64	4.4	4.26	2.25	3.41	9.3
3.15	1.64	2.60	4.0	4.26	2.28	3.49	9.3
3.16	1.56	2.61	4.6	4.27	2.13	3.53	9.1
3.16	1.56	2.71	4.2	4.31	2.27	3.46	10.1
3.17	1.64	2.57	4.2	4.38	2.35	3.47	11.0
3.19	1.57	2.67	3.7	4.45	2.38	3.48	9.5
3.20	1.66	2.70	4.2	4.45	2.39	3.56	10.3
3.24	1.69	2.75	5.0	4.47	2.40	3.67	10.5
3.25	1.70	2.73	5.1	4.60	2.44	3.67	12.0
3.26	1.65	2.67	4.7	4.80	2.53	3.69	13.6
3.27	1.69	2.63	4.5	4.84	2.57	3.96	15.2
3.29	1.76	2.75	4.4	5.02	2.58	3.98	15.7
3.35	1.78	2.78	5.0	5.16	2.66	4.15	14.6

ON THE HISTOGENESIS OF THE ISLANDS OF LANGERHANS IN *RANA JAPONICA* (GÜNTHER)

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(With 16 text-figures and Plate III)

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Since the completion of LAGUESSE's reports on the islands of Langerhans in the sheep embryo (1893, '94, 1905), embryological studies of the islands have been carried out by many investigators, using the embryos or larvae of various species as material: namely, PEARCE (1903), KÜSTER ('04), WEICHELBAUM and KYRLE ('09), MIRONESCU ('10), NAKAMURA ('24), and NEUBERT ('27) investigated the islands in the human embryo; HELLY ('06) those in the guinea-pig embryo; ARON those in the embryos of the sheep, guinea-pig, and man ('20, '20a, '20b, '22, '26, '31), and also in the tadpoles of *Rana temporaria*, *Rana esculenta*, and *Bufo vulgaris* ('25, '28, '28a, '31); POTVIN and ARON ('27) those in the chick embryo; VAN CAMPENHOUT ('25, '27) those in the embryos of the sheep, dog, mouse, man, daman, calf, and guinea-pig; LENTATI ('28, '29, '30) those in the embryos of *Mus musculus*, *Mus decumanus*, guinea-pig; and so on.

LAGUESSE distinguished the primordial island cells from the other pancreatic cells on the borders of the primitive pancreatic cords in sheep embryos 13–18 mm. long (*vide* ARON '22), by the criterion that the former cells stain more intensely than the latter. According to him, the primordial island cells are very precociously grouped into small masses, and when the primitive cords begin to form primitive pancreatic tubules (embryo 18.5 mm. in body length), the primordial cells increase in number, and form primary islands, which show the same cellular disposition and the same mode of vascularization as those of the adult, except that they are more intensely stainable. Some of the primary islands form groups with each other and fuse into larger ones. Later, in an embryo 90 mm. long, when the pancreatic duct system and acini are established, the primary islands cease to form, and then atrophy sets in. Meanwhile, the acinus cells surrounding the secreting cavities begin to be transformed into the secondary islands. Here and there, some adjacent secondary islands combine

with each other and form a larger one. LAGUESSE was of opinion that reversion between the acini and the secondary islands might be possible ('balancement theory').

Afterwards, some controversies arose about the mode of formation of the islands, especially in relation to the degeneration of all the primary islands, to the transformation of the fully differentiated acinus cells into the secondary island cells, and to the reversibility between the islands and the acini.

According to PEARCE, in the third month of the human fetus (54 mm. in body length), the islands of Langerhans originate in the cells of the primitive secreting tubules by proliferation and differentiation. At first the islands are continuous with the acinar processes, from which they originate, and then are gradually constricted off from the acini, as the development goes on. He opposes VON HANSEMAN's theory, which maintains that the islands develop from the cells of the connective tissue.

KÜSTER, WEICHELBAUM and KYRLE, and LENTATI invariably coincide in the opinion that the islands of Langerhans are formed from the duct system by budding. KÜSTER, however, seems to be of opinion that they are differentiated only from predestined cells, and never from ordinary duct cells.

MIRONESCU is sure of the fact that the first anlage of Langerhans' island is differentiated from a bud which has formed in the epithelium not only of the secreting duct but also of the acini. The embryo, in which he found the very earliest anlage of the island, was one 17-18 weeks old, the acini having already differentiated histologically, and the islands having maintained connection not only with the acini but also with the duct system.

VAN CAMPENHOUT is the first and hitherto the only authority who is of opinion that the sympathetic nervous system has an important relation to the histogenesis of the islands of Langerhans. He ('27) made a distinction between the two kinds of these islands: viz.

1) *Primary islands* or 'îlots de Laguesse (ARON '22).' The islands are formed in the walls of the primary pancreatic tubules. The cells of the islands grow out and migrate towards the sympathetic nerve ganglia or fibres until they come in close contact with the nervous elements. The latter then stimulate the primary island cells so that they are transformed into the secondary.

2) *Secondary islands* or 'îlots de Langerhans' The islands are transformed directly from the acinus tissue cells, not only without relation to

the sympathetic nervous elements but also without going through the primary stage.

In spite of these assertions of his in regard to the sheep ('25) and later ('27) also to the dog, mouse, man, calf, daman, and guinea-pig, the opinion of VAN CAMPENHOUT is not accepted by recent workers, namely NEUBERT, LENTATI, ARON, and so on.

NEUBERT published in 1927 a very valuable work, viz. a study of the pancreas, with reference to the 'Teilkörpertheorie' of HEIDENHAIN. He is of opinion that zymogenous and duct cells are equipotential, being able to transform one another, and both tissues being also able to give rise to the islands. The conclusion arrived at by him is merely deduced from his observation that the islands are in direct connection not only with the acini but also with the preterminal ducts, and that they increase in number. He believes the transformation of the islands occurs not in the whole acinus cavity, but in only a few cells of it, thus admitting first differentiation, and then proliferation.

LENTATI describes certain islands, which are continuous with the acini, and is of opinion that such a continuity has resulted only secondarily, and was not caused by the direct transformation of the acinus tissue into the islands.

From above views, it is evident that there are two theories, one that the formation of islands occurs in both the duct system and the zymogenous tubules; this theory is supported by LAGUESSE, MIRONESCU, VAN CAMPENHOUT, and others; and the other that the formation occurs only in the duct system as is held by PEARCE, KUSTER, HELLY, WEICHSELBAUM and KYRLE, NAKAMURA, LENTATI, and others. But they all, with the exception of ARON, agree on the point that some primary islands can undergo further differentiation into the functional islands. ARON ('22, '31) confirms LAGUESSE's work, and persuades himself into the belief that the 'îlots de Laguesse' in the sheep change finally into either 'hématiformation' or regression, and that the 'îlots de Langerhans' are formed from the acini by transformation or from the duct system by budding. ARON ('22) recognizes, however, the transformation of the primary islands into the secondary in the guinea-pig and in man.

The present work of mine was undertaken at the suggestion of Prof. Dr. E. NOMURA in the spring of the year 1932. The purpose of this study was initially to determine whether in the amphibian larvae the islands have some connection in origin with tissues other than the endoderm or not.

As to the development of the amphibian pancreas, the work of ARON and ALFONSI together ('24) and that of ARON alone ('25, '28, '28a, '31) have been referred to.

According to the former work, functional island cells have appeared very precociously in larvae about 10 mm. long, but the primary island cells (cellules de Laguesse) occur later during metamorphosis. The authors were of opinion that this is incidental to extra-uterine life from a very early stage of development. But ARON rejects this conception in his later work ('31) in the case of *Rana temporaria*, *Rana esculenta*, and *Bufo vulgaris*, and states that the primary islands appear very precociously and the secondary islands come first into view in the early stage of metamorphosis. Furthermore, he believes in the occurrence of another kind of islands, 'glandes de la metamorphose', which have been transformed from the 'canaux excréteurs' of the pancreas. The cells of the 'glandes de la metamorphose' are rich in cytoplasm, and show a clear appearance in the background of the darker zymogenous parenchyme cells.

Before entering on the subject of this paper, I wish to express my deep gratitude to Prof. Dr. E. NOMURA, to Assist. Prof. I. MOTOMURA, and to Mr. J. OIZUMI for their kind guidance during the progress of my work and also for their kindness in collecting the scientific publications on the subject.

MATERIAL AND METHOD

The larvae of *Rana japonica* were selected as the material for investigation. The fixation of the material was begun 2 days after the hatching and repeated at intervals of every 3 days for 20 days and then at desired stages till the complete absorption of the tail. Moreover, the pancreas of the adult frog was also preserved for comparison with those of the larvae. The serial sections were made of a thickness of 8 μ .

For the fixation, Lane's A and B solutions, Perenyi's, Bouin's, and Champy's fluids were tested, and for the staining, Delafield's hematoxylin and eosin, Heidenhain's hematoxylin, and Mallory's connective tissue stain were also tried.

The alcoholic and acidic fixing solutions were not satisfactory, because of their property of dissolving the plasmic contents in zymogenous cells, this frequently making the observation more difficult.

Lane's B solution (corrosive sublimate 5 gm., potassium bichromate 2.5 gm., and distilled water 100 cc.) combined with Mallory's connective tissue stain was fairly effective. This method revealed very fine colour

differentiation: *i. e.* in the metamorphosis stages, in which the histological differentiation of the pancreas has been fairly established, the zymogenous cell-plasm takes on a blue colour, the primary island cells dark purple. The secondary or adult island cells are usually clearer than the primary, and show varying depth of the staining colour according to amount of the characteristic dark purple granules contained, while the preterminal duct cells and centro-acinus cells appear very clear without containing any such granules.

OBSERVATIONS

In describing my observations, the sequence of the developmental stages is followed from the earlier stage to the later. The body length given as to the respective stage is only approximate.

1) Stage reached 2 days after hatching (7 mm. in body length).

The pancreas appears as a mass of cells. Each cell is nearly spherical, and contains so many yolk granules that the large nucleus is more or less irregularly compressed among them. The cytoplasm is sparse, and remains almost unstainable. Most of the nuclei contain, respectively, a large nucleolus. Among these ordinary cells we find some peculiar cells, each of which contains small yolk granules, abundant melanin pigments, and a nucleus with two comparatively small nucleoli (Fig. 1).

As will be seen later, in the later stages the island cells, centro-acinus cells, and the zymogenous cells come to be distinguished, respectively, as follows:

a. In the island cells, the nucleus contains one or two small nucleoli and a fine linin network.

b. In the centro-acinus cells, the nucleus contains one or two small nucleoli and a coarse linin network.

c. In the zymogenous cells, the nucleus contains one or two large nucleoli and a coarse linin network.

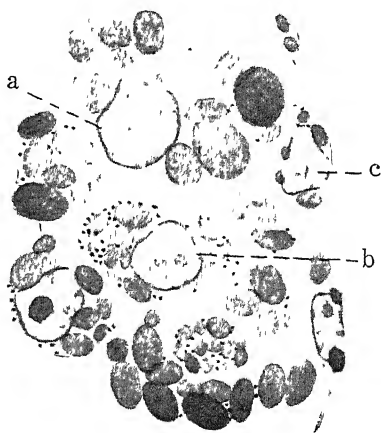


Fig. 1. Section of pancreatic mass in stage 2 days after hatching. *a* nucleus containing large nucleolus, *b* nucleus containing two small nucleoli, *c* nucleus of peritoneal cell. $\times 1060$.

Thus even in the present stage, the cells which have a nucleus containing one or two large nucleoli and a coarse linin network may be identified as the predecessors of the zymogenous cells, while those cells with a nucleus containing small nucleoli are not to be identified as those of either the island cells or the centro-acinus cells, owing to the absence of any distinct criterion which can be applied to the linin network. It appears to me, however, to be most probable that the cells containing comparatively abundant melanin pigments are destined to become the island cells.

2) Stage 5 days after hatching (8 mm. in body length).

Cellular components of the pancreas come into close contact with each other and no longer show the roundish appearance. Demarcation lines which separate the components become obscure. Cytoplasm is somewhat basophile, showing a little darkish colouration and is yet sparse in the cells of the central part of the pancreas, while it is more densely contained in those of the peripheral. In most peripheral parts of the pancreas there appears the first sign of the lobulation, a number of central lumens being established independently of each other as discontinuous spaces. The polarity of the cells is already defined, so that the yolk granules are disposed towards the outer or basal side of the cells, and the comparatively dense cytoplasm towards the inner side (Fig. 2).

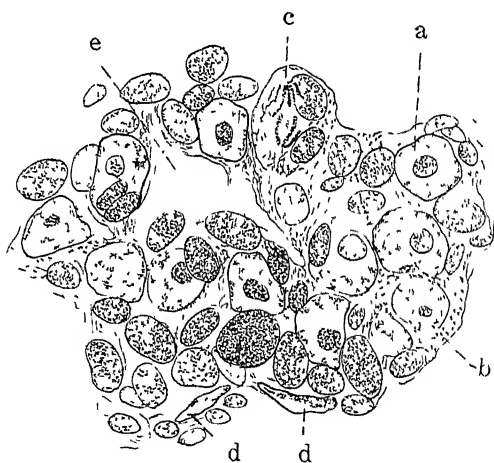


Fig 2 Section of lobulating portion of pancreatic mass in stage 5 days after hatching. *a* nucleus containing large nucleolus, *b* nucleus containing small nucleolus, *c* nucleus in division, *d* nucleus of peritoneal cell, *e* central lumen. $\times 1060$.

The primary island cells are found singly in most cases, being scattered among the primordial zymogenous cells in either the lobulated (Fig. 2) or non-lobulated (Fig. 3) portion of the pancreas, particularly in its peripheral region. The zymogenous cell can be easily distinguished from the island cell by the presence of a nucleus which is furnished with one or two large nucleoli, coarse linin network, and a thick nuclear membrane, and by the presence of the cytoplasm which is somewhat basophile, being darkish when observed, though it does not yet show in this stage any indication of zymogen granules at all. In each island cell, the cytoplasm is clear and almost unstainable, being packed with abundant very fine melanin pigments and with yolk granules, which are generally smaller in size and fewer in number than those in the zymogenous cells, and are rarely completely lacking (Figs. 2*b* and 3*b*). The nucleus is furnished with one or two much smaller nucleoli, much finer linin network, and a thinner nuclear membrane than those of the zymogenous cell, respectively (Fig. 2). In the hematoxylin preparations of the present stage, it is revealed that the chromatin granules are fewer in the nuclei of the island cells than in those of the zymogenous ones.

In the peripheral part, where the pancreatic tissue has begun to lobulate, we sometimes find blood corpuscles, which have probably invaded it, as the formation of blood capillaries goes on into the interspace between the lobulated tubules, even though the capillary walls have not yet come into view owing to the weakness of the staining reaction.

3) Stage 8 days after hatching (9 mm. in body length).

The lobulation process has almost got into the central part of the pancreas, but it is yet very irregular at present there. Zymogenous granules are deposited in the cytoplasm along the central cavities. In each zymogenous cell the yolk granules are yet retained in abundance, especially in the proximal or basal part of the cells. The cytoplasm is not yet thoroughly basophile. The

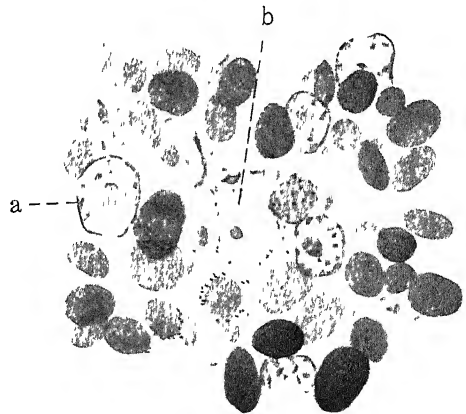


Fig 3. Section of non-lobulated portion of pancreatic mass in stage 5 days after hatching. *a* nucleus containing large nucleolus, *b* nucleus containing small nucleolus. $\times 1060$.

centro-acinus cells are just perceptible, and are scattered here and there in the zymogenous tubules.

The primordial islands are found scattered among the zymogenous cells along the blood capillaries, being composed, in most cases, of single cells, and being very rarely as many as five or so. The island cells are generally very poorly furnished with cytoplasm. The yolk granules become smaller and fewer than those in the preceding stage, but the melanin pigments are so abundant, that the appearance of the cytoplasm is in every case obscured by their presence. As a result of using the preparations of Mallory's connective tissue stain, a conspicuous colour differentiation is observable in the cytoplasm of the island cells, some taking on a dark purplish colour, others being almost unstainable, and others again showing various tinges between the two. This transition of the colour is gradual, but owing to the difference in colour the island cells are always distinct from the zymogenous cells which usually take on a blue colour (Fig. 4).

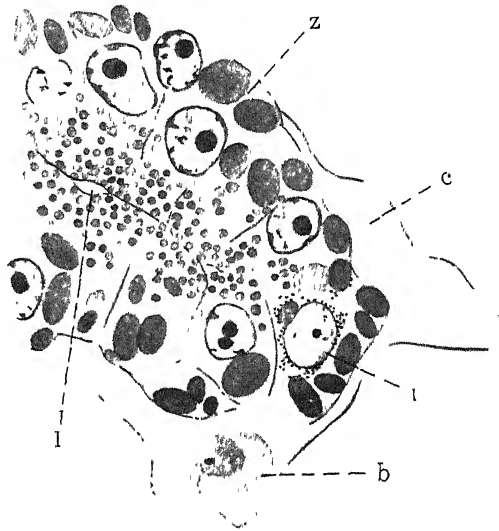


Fig. 4. Section of pancreatic tubule in stage 8 days after hatching, to illustrate difference of behaviour between island and zymogenous cells *b* blood corpuscle, *c* blood capillary, *i* island cell furnished with a few yolk granules, abundant melanin pigments which surround two or more clear, unstainable, ovoidal areas probably occupied formerly by yolk granules; nucleus containing small nucleolus, and cytoplasm staining proximally in dark purple, *l* acinus cavity, *z* zymogenous cell furnished with zymogen granules distally and large yolk granules proximally, and with nucleus containing large nucleolus. $\times 1060$

A few primordial or primary islands are often met with, arranged facing the course of the same blood capillary.

4) Stage 14 days after hatching (11 mm. in body length).

The features of the primary island cells are nearly the same as those of the preceding stage, but showing very often a tendency to become round (Fig. 5).

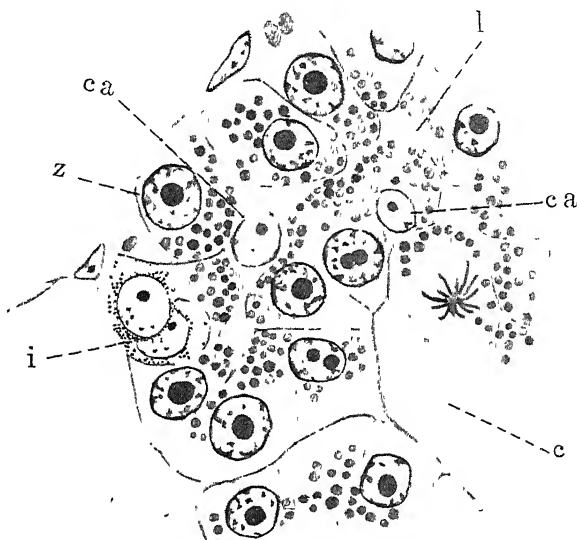


Fig 5 Section of zymogenous tubule in stage 14 days after hatching, to show island cells becoming rounded distally *c* capillary, *ca* centro-acinus cell, *z* island cell, *l* acinus cavity, *z* zymogenous cell $\times 1060$.

5) Stage 20 days after hatching (12 mm. in body length).

The yolk granules have almost completely disappeared. The island cells show nearly the same features as those in the preceding stage, with the exception of the absence of the yolk granules.

The islands are as yet very inconspicuous, being composed only of a single or a few cells. They make small masses of about 22μ in the largest diameter but very rarely. In such masses the nuclei are very closely assembled, and irregularly arranged with each other, and the demarcation lines of the cell components are indiscernible.

6) Stages of posterior limb-buds (25-30 mm. in body length).

The histo-physiological differentiation of the zymogenous cells has already been completely established, the cytoplasm showing a decidedly

basophile reaction. The excretory function is revealed by a varying amount of zymogen granules in the cell bodies and by the existence of excreting fluid in the central lumen.

The primary island cells remain almost unchanged, the nuclei showing the typical structure, but the cytoplasm taking on the dark purplish tinge more prominently, and the melanin pigments becoming coarser in size and fewer in number. They sometimes form small round masses, and sometimes show an epithelial arrangement of a height only a little more in measurement than the longest diameter of the nuclei, which are ovoidal or more or less elongated (Fig. 6).

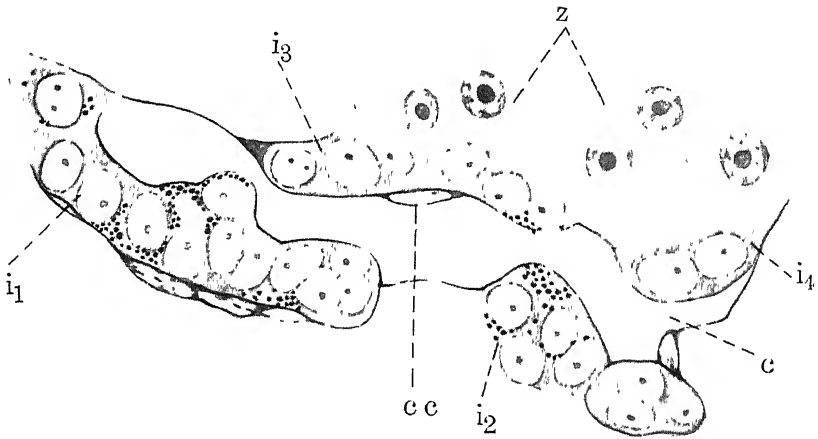


Fig 6 Section of pancreas in later stage of posterior limb-buds, to show state of aggregation of island cells around blood capillary c blood capillary, cc nucleus of capillary cell, i island cells, z zymogenous cells $\times 1060$.

Some single primary island cells containing coarse melanin pigments are found here and there, forming completely unique acini together with zymogenous cells (Fig. 7).

One (*id*) of the two islands shown in Fig. 7 is perhaps composed of three cells, two nuclei of which are about to degenerate. Some islands in the same stage are found disposed with nearly all the surface facing the capillary and with the small surface facing the central lumen, as if the islands were bulging out into the capillary (Fig. 8).

Cases, in which some primary islands surround a same capillary, become very frequent (Fig. 6). The islands i_1 , i_2 , i_3 , and i_4 are really connected with each other in the other serial sections. The present island

was the most developed of those which I have been able to find so far in this stage, measuring about 104μ by 30μ .

7) Stage in which the posterior limbs have already become articulated and the buds of digits are discernible (4–5 mm. in length of posterior limb, and 35 mm. in body length).

Most primary islands which are composed of many cells, retain the characteristic feature described in the preceding stage. The cell components are poorly furnished with cytoplasm, and show a dark purple reaction to Mallory's connective tissue stain. They retain also a number of coarse melanin pigments, but sometimes lack them entirely.

It is worthy of note that, in the present stage, some of the unicellular islands and of the smaller islands, which are composed of a small number of cells, begin to undergo a

very conspicuous change in staining reaction, while most of them, as well as the typical primary island cells, retain the original characteristics. The former island cells alter in character from basophile to intensely fuchsinophile, transitional colourations between the dark purple and the red having been frequently met with. Most of the single fuchsinophile cells are of nearly the same size as the other primary island cells, which are much smaller than the zymogenous cells. When they constitute unique tubules together with zymogenous cells, they are inclined frequently to be rounded, as is the case in the unicellular primary island cells. Sometimes, the densely fuchsinophile cells are found among the cells forming larger islands, especially in their peripheral part, where they are more closely

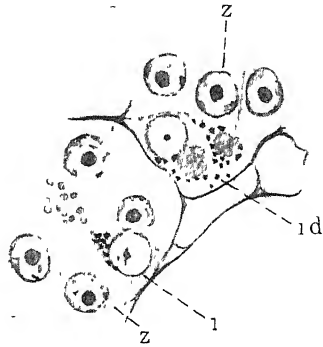


Fig. 7. Section of pancreas in later stage of posterior limb-buds, to show island cell forming a unique acinus together with zymogenous cells *c* blood capillary, *i* and *id* island cells, *z* zymogenous cells $\times 1060$

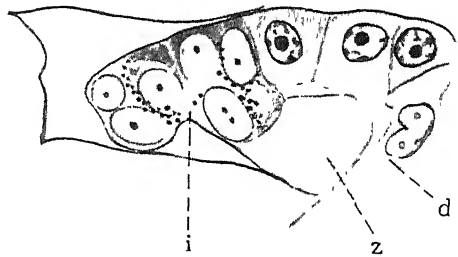


Fig. 8. Section of pancreas in later stage of posterior limb-buds, to show island nearly surrounded by blood capillary. *d* preterminal duct cell, *i* island, *z* zymogenous cells $\times 1060$

attached to the zymogenous tissue (Fig. 13if).

There are various types of fuchsinophile cells. In most of them the content is not homogeneous owing to the presence of the fuchsinophile granular structure. Sometimes the granular structure is not distinct (Fig. 9A and B, and Pl. III, Fig. 1A), but sometimes it is very distinct (Fig. 9C). In a few cells, which are richly furnished with cytoplasm, the content is quite homogeneously fuchsinophile, being feebly stained and never showing granulations (Fig. 9D, and Pl. III, Fig. 1D). One or two notches in the nuclear membrane are found in some of the fuchsinophile cells (Fig. 9A and B, and Pl. III, Fig. 1A). Henceforth, all the cells, which take on the fuchsin stain, are called invariably the 'fuchsinophile cells.'

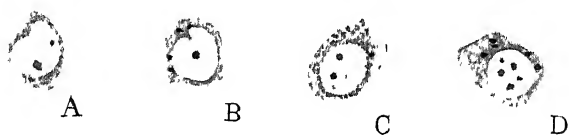


Fig 9 Fuchsinophile island cells, to show several types. A and B cells in which fuchsinophile granules are not distinct, C cell in which fuchsinophile granules are distinct, D cell which is homogeneously fuchsinophile, showing no granulations, but containing a thread-like basophile substance. $\times 1060$.

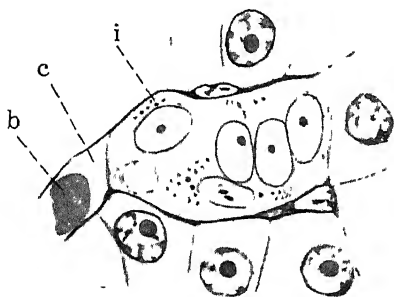


Fig 10 Section of pancreas, to illustrate most advanced differentiation of island cells in stage of body length of 35 mm. b blood corpuscle, c blood capillary, i island cells. $\times 1060$.

In the present stage, another transition begins to occur in some primary island cells to the secondary. Some primary island cells become higher and columnar, being richly furnished with clear cytoplasm, and the purple granulation comes into view in the preparations with Mallory's connective tissue stain (Fig. 10).

8) Stage in which the posterior limbs are highly developed and actively movable (12 mm. in length of posterior limb, and 45 mm. in

body length).

In most of the islands, the cytoplasm becomes voluminous, and the purple granulation clearly detectable, but in some of the islands the granular content of the cytoplasm is so sparse that the cells are very

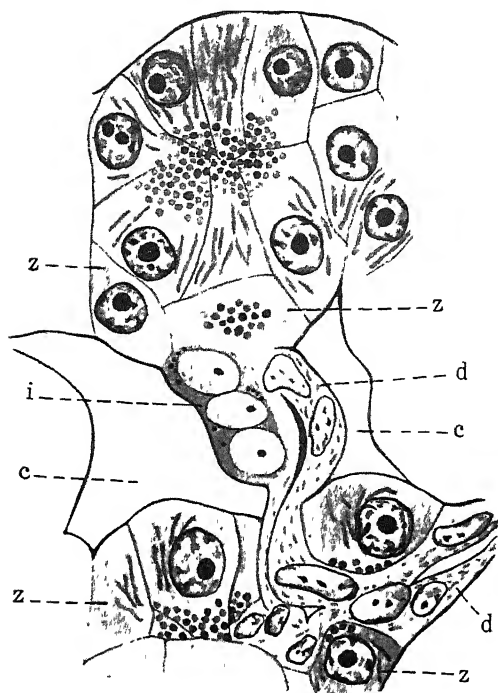


Fig. 11. Section of pancreas in stage of body length of 35 mm. to show island decisively in close continuity with preterminal duct. *c* blood capillary, *d* preterminal duct, *i* island cells, *z* zymogenous cells. $\times 1060$.

clearly recognizable. A small number of melanin granules are also usually found. The cell components are often spindle-shaped or columnar, but are sometimes short, and often arrange themselves in a band (Fig. 12). These features are directly those of the secondary or adult island cells. The primary islands are still observable.

The fuchsinophile cells show varying relations to the zymogenous tubules as do the primary island cells. They are sometimes large and sometimes as small as the latter cells; sometimes compose, singly or forming masses of a few cells, unique acini together with the zymogenous tissue, and sometimes they are found intercalated between the outer surface of the zymogenous tissue and its basement membrane. In a few of the fuchsinophile cells the melanin pigments still are retained in abundance, while in most of them they are almost lacking. Frequently, some of the fuchsinophile cells are found in direct continuity with clear,

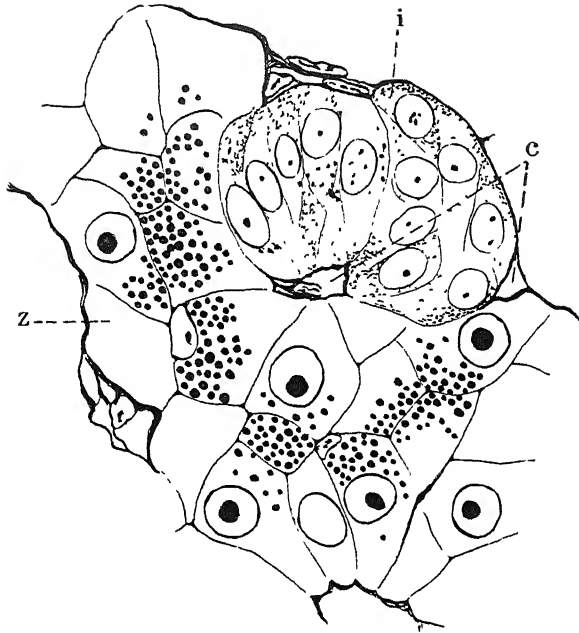


Fig 12. Section of pancreas in stage of body length of 45 mm., to show arrangement of cellular components of secondary island forming band, which measures about 23μ in width. *c* blood capillary, *i* secondary island, *z* zymogenous cells $\times 1060$.

secondary island cells. In these cases, they are located generally in the periphery of the island adjacent to the zymogenous tissue, as described in the preceding stage (Fig. 13).

In the stage now reached, it is evident that some primary island cells and fuchsinophile cells undergo degeneration. In one case met with, an island was composed of 7 cells (Pl. III, Fig 2). One (*if*) of the cells was deeply fuchsinophile. Three others (*is*) were on the way to differentiation into the secondary cells. And the remaining three (*ip*) were primary island cells beginning degeneration, their nuclei being diminished in size and darkly stained. The island in this case was in the most intimate 'continuation' with the zymogenous tissue, but any evidence of transition from zymogenous cells to island cells, or *vice versa*, was not observable.

The largest island met with in this stage had attained nearly $100\mu \times 100\mu \times 40\mu$, and the histological differentiation had been almost completely advanced with rich blood supply.

From about this stage onwards there occurs in the pancreas a distinct reorganization process. The pancreas gradually decreases in size and

alters from a massive to a slender form, until the metamorphosis is complete. It may be emphasized here that the island cells, either the secondary or the deeply fuchsinophile cells, begin to atrophy later than do the zymogenous cells, for I frequently met with secondary islands and fuchsinophile cells freed from zymogenous tissue by the atrophy of the cells of the latter. In most cases, the liberated fuchsinophile cells are homogeneously stainable. Their shape becomes rounded first, and then begins to be disfigured showing signs of degeneration.

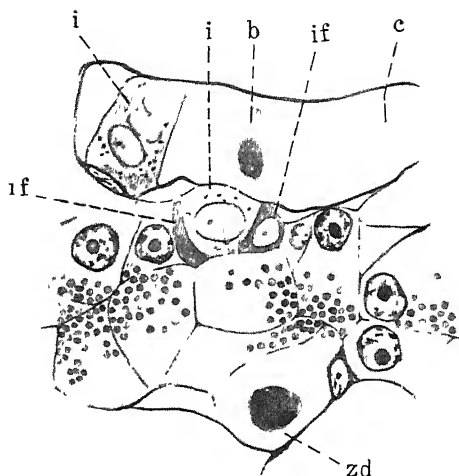


Fig 13 Section of pancreas in stage of body length of 45 mm., to show fuchsinophile cells in periphery of island, being attached to zymogenous tissue. *b* blood corpuscle, *c* blood capillary, *i* secondary island cells, *if* fuchsinophile cells, *zd* degenerating zymogenous cell $\times 1060$

REMARKS

1) General. In the stage 2 days after hatching, I was not able to find any primary island cells, yet some cells (Fig. 1) may be explained as the primary island cells, as they contain more abundant melanin pigments and smaller nucleoli than do the other cells. In the larvae, 5 days after hatching, the primary island cells were distinctly discernible for the first time among the parenchyme cells, which in this stage mainly form the primitive pancreatic mass, just beginning differentiation as the zymogenous cells. Most of the primary island cells in this stage contain yolk granules as do the other parenchyme cells, but the amount of fine melanin pigments is distinctly richer in the former cells than in the latter. The nucleus of each primary island cell, furnished with one or two small nucleoli and fine linin network, shows the features of that of the typical island cell in the adult. But when observed in hematoxylin preparations, the amount of chromatin granules is discordantly poor in the nuclei of the primary island cells. From the fact that these primary island cells are found even in the non-lobulated parts where no capillaries are developed (Fig. 3), I conclude that, in their origin, they have nothing directly to do with the

capillary or connective tissue cells already differentiated. This view is in opposition to that of VON HANSEMANN (1901, *vide* PEARCE '03). Again from the fact that the cells, which show a similar behaviour not only to the mesenchyme but also to the peritoneal cells, are never found among the cell components of the primitive pancreatic mass (Fig. 1), I therefore feel bound to conclude that the primary island cells are endodermal in origin as are the other pancreatic parenchyme tissues.

When the lobulation begins, the primary island cells come into close connection with the zymogenous tubules, and also with the blood capillaries which have been formed round the zymogenous tubules as the lobulation proceeds (Fig. 4). The primary island cells are scattered for the most part in the peripheral region of the pancreas (Fig. 14). They are interposed between zymogenous cells, thus showing the unique acinus formation together with the zymogenous tissue (Figs. 4 and 5) or intercalated between zymogenous tissue and its basement membrane, which is in most cases nothing but capillary walls. The cytoplasm of the primary island cells begins gradually to show the typical staining, that is, a dark purple tinge in the case of Mallory's connective tissue stain (Pl. III, Fig. 1*z*), and a

light red in that of Delafield's hematoxylin and eosin stain. Chromatin granules become more abundant.

Many authorities are of opinion that the primary island cells migrate from their original situation in the zymogenous tissue outwards to the connective tissue. This may possibly be the case. I have been able to find, as already stated, the primary island cells interposed between the zymogenous cells (Fig. 5) and those intercalated between the zymogenous tissue and its basement membrane (Fig. 6*i*₄), just showing a movement from one situation to the other. This movement of the cells, however, is, in my opinion, not due to their automatic migration, but to the transference caused by a difference of tension between

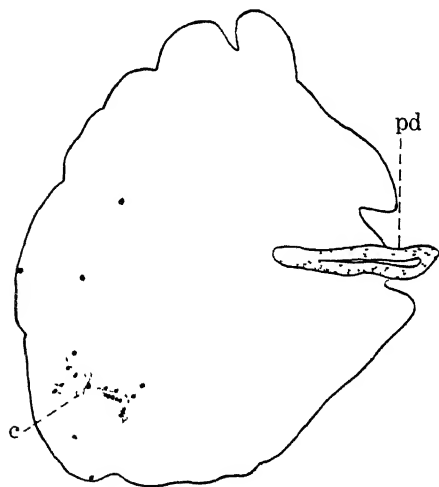


Fig. 14. Cross section of pancreas in stage 11 days after hatching through ductus pancreaticus, to illustrate the state of distribution of the primary island cells. *c* blood capillary, *pd* ductus pancreaticus

the membrane of the zymogenous cells and the basement membrane, which is directly the wall of the blood capillary. Moreover, in the cases which I investigated, neither tropism of the primary island cells towards, nor other topographical relations with, the sympathetic nervous system, could ever be met with.

The primary islands retain their present features until the metamorphosis begins. In the stages in which posterior limb-buds develop, the primary islands are found sometimes as single cells, sometimes as small concretions composed of a number of cells which are arranged either, irregularly, forming rounded masses (Fig. 6i₂) or, regularly, forming bands (Fig. 6i₁). The gradual transition of the primary islands into the secondary is carried on from this stage onwards to the stage in which the posterior limbs are actively movable (45 mm. in body length). In the latter stage, the islands which are composed of cells identical with the typical island cells of the adult are commonly found. As to the generative cause of this transition, I have nothing to oppose to the theory put forward by ARON (1926, '28a, '31), that the differentiation into the secondary island cells is preceded by the histo-physiological differentiation of the thyroid. Several primary islands are frequently found round the same capillary in close connection with the neighbouring acini, as is described by almost all authorities. When these islands grow along the capillary and fuse themselves with each other to form a larger island, the one capillary becomes divided into many, some being taken into the island. It is clear that the capillary walls of the island are homologous with the basement membrane of the zymogenous tissue as described by FISCHER (1912).

2) So-called 'capsule' of the island. According to PEARCE, KÜSTER, and WEICHSELBAUM and KYRLE, the islands are originally continuous with the other pancreatic tissue, and later, as the development goes on, they are constricted and thus separated from the latter, the former being thus surrounded completely by the 'capsule' of the connective tissue. They state also that such an anatomical independence of the islands from the other pancreatic tissues coincides with the fact that the former are independent of the latter in physiological function. Recent authorities, NAKAMURA, NEUBERT, and LENTATI, however, deny that the existence of such complete capsules is invariable. According to FISCHER, and SAGUCHI, and to my own observation on the pancreas of the frog, the islands are clearly functional even without the capsules, which separate the islands from the other pancreatic tissues.

3) Fuchsinophile cells. These cells are very rare in the early stages

of metamorphosis, and gradually increase in number as the metamorphosis advances.

The fuchsinophile cells of mine are nothing else but those which are found by SAGUCHI in the pancreas of the frog, being explained as transitional forms from the zymogenous cells to the island cells, or *vice versa*. These cells are also identical with the 'bathychrome cells' of VINCENT and THOMPSON (1908) found in the pancreas of several kinds of species of Vertebrates. BENSLEY's a-cells in the pancreas of the guinea-pig are also the same as my fuchsinophile cells. According to BENSLEY, the a-cells are present not only in the periphery of the island adjacent to the acini, but also in the central part of the island. He says that this may cause one difficulty to the authorities who consider these cells to be those which represent a transitional stage from the acinus cells to the island cells. I was able to find, as already described, in the early stages of metamorphosis, various cells which show a gradual change in the staining reaction from dark purplish tinge to deeply fuchsinophile. I have also stated that these cells occur, either singly or grouped in small masses of a few cells, closely attached to the acinus cells in the periphery of an island. These facts appear to me to be very suggestive, and I wish to deduce here that some of the primary island cells degenerate and some become fuchsinophile cells, while they are in contact with the acinus cells; but most of them, especially the components of large primary islands, become the secondary or functional island cells.

According to ALFONSI, the 'cellules de Laguesse', which were found by him in the tadpoles during metamorphosis, begin to appear later than the appearance of the 'cellules de Langerhans.' Therefore, his 'cellules de Laguesse' are not actually the same as the 'cellules de Laguesse' of ARON, which are identical with the primary island cells and begin to appear before the appearance of 'cellules de Langerhans'; and according to his statement they are, without doubt, nothing but the fuchsinophile cells.

4) New formation of the island from other tissues. The view that there is a new formation of the islands from the other tissues, even in newly born animals, has been held by many authorities. According to WEICHSELBAUM and KYRLE, NAKAMURA, and LENTATI, etc., the islands which have been retained in close connection with the duct system, and, according to ARON, VAN CAMPENHOUT, NEUBERT, and others, those in close connection with the zymogenous tubules, are newly-differentiated and proliferated ones. Most of the authorities, however, seem to have arrived

at these conclusions from the fact that the islands increase in number during later developmental stages, and that they are in direct continuity with the duct and zymogenous tissues. But continuity with the other tissues appears to me to be insufficient to prove the occurrence of a new formation of the islands from the other tissues. The only way to decide this question is to find a direct case of transformation from one to the other.

Among these authorities, VAN CAMPENHOUT alone in his description refers to this mode of transition. But he merely states in regard to the mouse that "*chez l'adult, au contraire, à côté des complexes sympathico-insulaires bien délimitées, existent des îlots en rapport avec les acini et montrant toutes les figures de transformation d'acini en îlots*" Such a description is not sufficient to convince us that his transition theory must be accepted as proved. VINCENT and THOMPSON also believe in the occurrence of transition even in the adult pancreas. Taking the abundance of zymogen granules as the criterion, they state that zymogenous cells gradually "shade off" into island cells, and that "the transition as indicated by varying amount of zymogen granules in the different cells" is frequent. It seems to me, however, that the variation in the amount of zymogen granules gives no proof by which we may account for the transition from zymogenous cells to the island cells. We ought rather to look for positive cytological grounds of investigation as carried out by SAGUCHI in 1920.

VAN CAMPENHOUT, in his study of the pancreas of the dog, found that all the functional islands in the embryonic stages are in connection with the sympathetic nervous elements, but that in the adult some of the islands are transformed from acini, and do not show any such connection. Now, if in the embryonic stages, all the islands are really in connection with the sympathetic nervous system, no single primary island cells remaining scattered among the zymogenous cells, and yet later in the adult stages there are found many islands unconnected with the sympathetic nervous elements, but in connection with the zymogenous tubules in close 'continuity' with those elements, then it may safely be concluded that a new formation of the island of Langerhans in the acinus tissue is possible. However, I found no such connection between islands and sympathetic nervous elements in any stages of development. Consequently, I cannot agree with the view of VAN CAMPENHOUT.

NEUBERT believes in the possibility of the formation of the islands from both duct system and zymogenous tissue. But according to NAKAMURA and LENTATI, the connection between islands and zymogenous tubules is

of secondary formation. They assert that such a connection never involves proof of the direct origin of the islands, but they believe that the formation of the islands is only possible by budding in the duct system, observing the direct continuity between the preterminal ducts and islands and similar features in the components of the two tissues, perhaps in eosin preparations. In spite of BENSLEY's opinion that the continuity between the two tissues never proves the possibility of their reciprocal transition, many later authorities are accustomed to trust too much to the importance of continuity between the islands and other tissues.

Why did not these authorities identify the islands, which were in direct continuity with the duct system, with the islands derived from the primary island cells, which had differentiated in a very early stage in the walls of the primitive pancreatic cords or tubules, and which had been shown for the first time by LAGUESSE in the embryos of the sheep and then by HELLY in the embryos of the guinea-pig? Why did they not consider that the connection between the island and the duct system is also of secondary formation? Such an explanation, which makes the connection of the islands with either the zymogenous tubules or the duct system to be of secondary formation, seems to be highly acceptable when we compare this circumstance in the case of mammals and in that of frogs: in the former, the connection of the islands is retained more frequently with the duct system, while in the latter it is retained almost conclusively with the zymogenous tissue.

One case, which was very rarely found in the range of my observations and in which the island maintained close connection with the duct system, is shown in Fig. 11. This case, however, does not compel us to conclude that the differentiation of the islands occurs in the cells of the preterminal duct, as is asserted by various workers.

Early in the process of development, the pancreas is almost completely filled with zymogenous tubules, the cells of which have been differentiated histologically *pari passu* with the lobulation of the primitive pancreatic mass, which contains scattered centro-acinus and primary island cells; and the duct system consists of a single tube without any branches, and is confined to a very restricted area near its exit, ductus pancreaticus, only extending to a few sections, while the pancreas extends to about 70 sections (Fig. 14). The development of the duct system is practically accomplished in the tadpole after the metamorphosis has begun. In fact, in the development of the duct system two processes may be distinguished, as pointed out by NEUBERT: viz.

a. Development of the duct system by the growth of new branches from the primitive pancreatic duct.

b. Formation of preterminal ducts from centro-acinus cells. In this case, the centro-acinus cells proliferate and gradually line the acinus cavity (Pl. III, Fig. 1*ca*). Then the basement membrane grows into the

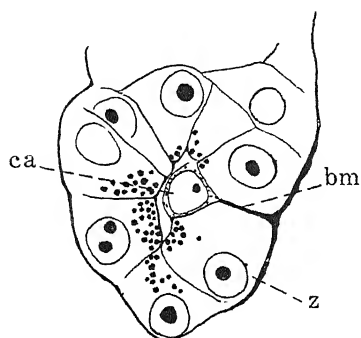


Fig 15 Section of zymogenous tubule in stage of body length of 25 mm, to show ingrowth of basement membrane *bm* basement membrane, *ca* centro-acinus cell, *z* zymogenous cell

interspace between the zymogenous cells and reaches the centro-acinus cells (Fig. 15, and Pl. III, Figs. 1 and 3), the zymogenous tubule being thus constricted and separated gradually into two (Pl. III, Fig. 3). These two portions of the zymogenous tubule, which have been constricted off, are separated by the further proliferation of the centro-acinus cells. Thus these cells form a bridge which is the preterminal duct (Fig. 11). Similar cases are frequently met with in the pancreas of the tadpoles in the early stages of metamorphosis.

If such an ingrowth of the basement

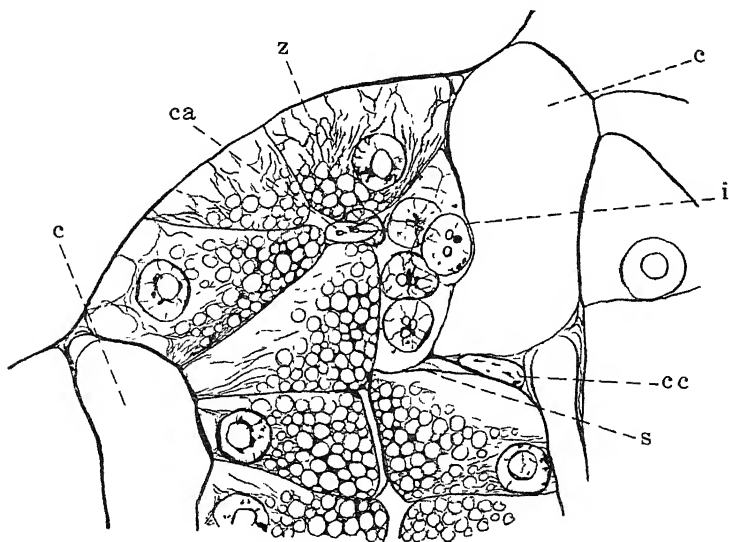


Fig 16. Section of zymogenous tubule in stage of body length of 25 mm., to show detachment (*s*) between primary island and zymogenous cell. *c* blood capillary, *ca* centro-acinus cell, *cc* nucleus of capillary cell, *i* primary island, *z* zymogenous cell.

membrane and further proliferation of the centro-acinus cells occur in the very portion of zymogenous tubule, where a primary island is laid (Fig. 16), it may be possible to form an island showing close connection with the preterminal duct (Fig. 11). At any rate, I cannot rely on the importance of the 'continuity' of the island with the other tissue, whether it be that of the duct system or of the acinus tissue.

SUMMARY

1) The primary island cells are differentiated from the cells of the same origin as the other pancreatic parenchyme cells, *i. e.* endodermal.

2) The primary island cells have in their origin nothing to do directly with capillary or connective tissue cells.

3) In the early stages of metamorphosis, the primary islands begin to differentiate into the secondary and become functional.

4) In the early stages of metamorphosis, the primary island cells, which are in the periphery of the island, and some primary islands, which are composed of only a single or a few cells are subjected to the other process of differentiation and become deeply fuchsinophile, while they are in close contact with zymogenous tissue.

5) The 'bathychrome cells' of VINCENT and THOMPSON, the 'a-cells' of BENSLEY, the 'a-, b- and c-cells' of SAGUCHI, and the 'cellules de Laguesse' of ALFONSI are included in the term 'fuchsinophile cells.'

6) The continuation between the islands and the other tissues does not involve proof of the actual origin of the islands.

7) The complete capsule which separates an island from other pancreatic tissues is not necessary for the physiological independence of each other.

LITERATURE CITED

- ARON, M. 1920. Hématiformation dans les îlots de Langerhans du pancréas embryonnaire. C. R. de la S. de Biol., 83.
- ARON, M. 1920a. Sur le développement des îlots de Langerhans fonctionnels dans le pancréas embryonnaires. Ibid., 83.
- ARON, M. 1920b. De la concomitance entre l'apparition des îlots de Langerhans fonctionnels chez l'embryon et l'établissement de la fonction glycogénique du foie. Ibid, 83.
- ARON, M. 1922 L'évolution morphologique et fonctionnelle des îlots d'endocrines du pancréas embryonnaire. Arch. d'Anat, d'Hist. et d'Embryol., 2.
- ARON, M. 1925 Dégénération du pancréas au cours de la métamorphose chez *Rana esculenta*. Régénération consécutive de l'organe. C. R. de la S. de Biol, 93
- ARON, M. 1926 Evolution de la thyroïde foetale chez les Mammifères. Sa concordance avec l'évolution du pancréas endocrine. Ibid., 94.

- ARON, M 1928 Le fonctionnement du pancréas chez les larves d'Amphibiens Ibid, 99.
- ARON, M. 1928a. Corrélation fonctionnelle entre la glande thyroïde et le pancréas endocrine chez les larves d'Amphibiens Ibid., 99.
- ARON, M. 1931 Recherches histo-physiologiques sur le fonctionnement et les corrélations des glandes endocrines embryonnaires chez les Vertébrés. Bull. Biol, 65.
- ARON, M et ALFONSI, N. 1924 Recherches sur l'histogenèse et la physiogenèse comparées des îlots pancréatiques endocrines des Batraciens C. R. de la S de Biol, 91
- BENSLEY, R R. 1911. Studies on the pancreas of the guinea pig Amer Journ. Anat., 12
- CAMPENHOUT, E van 1927. Contribution à l'étude de l'histogenèse du pancréas chez quelques Mammifères les complexes sympathico-insulaires Arch de Biol, 37.
- FISCHER, H 1912 Über die Langerhansschen Inseln im Pankreas von Amphibien Arch Mikro Anat, 79
- HELLY, K. 1904. Studien über Langerhanssche Inseln Arch. Mikro Anat Entw, 67.
- KUSTER, H 1904 Zur Entwicklungsgeschichte der Langerhansschen Inseln im Pankreas beim menschlichen Embryo Arch Mikro Anat Entw.-Gesch, 64.
- LANE, M A. 1907 The cytological characters of the areas of Langerhans. Amer Journ. Anat, 7
- LENTATI, G 1928 Ricerche sulla istogenesi delle isole del Langerhans. Rendic della R. Acc. Naz. dei Lincei, 7.
- LENTATI, G 1929 Ricerche sulla istogenesi delle isole del Langerhans in "*Ovis aries* L" Ibid, 9.
- LENTATI, G 1930 Primo contributo allo studio del tessuto endocrino primario del pancreas degli uccelli. Ibid, 11.
- LENTATI, G 1930 Ricerche sulla istogenesi delle isole del Langerhans in alcuni Mammiferi Arch. Ital. Anat Embryol., 28
- MIRONESCU, T. 1910 Über die Entwicklung der Langerhansschen Inseln bei menschlichen Embryonen Arch Mikro Anat, 76.
- NAKAMURA, N. 1924. Untersuchungen über das Pankreas bei Föten, Neugeborenen, Kindern und im Pubertätsalter. Virchow Arch, 253.
- NEUBERT, K. 1927. Bau und Entwicklung des menschlichen Pankreas. Beitrag 7 zur synthetischen Morphologie Arch Entw-Mech, 111.
- PEARCE, R 1903 The development of the islands of Langerhans in the human embryo Amer. Journ. Anat, 2
- VINCENT, S and THOMPSON, F D 1908 On the relations between the "islets of Langerhans" and the zymogenous tubules of the pancreas. Intern. Monatsschr. Anat. Physiol, 24.
- SAGUCHI, S 1920 Cytological studies of Langerhans islets, with special reference to the problem of their relation to the pancreatic acinus tissue. Amer Journ. Anat., 28.
- POTVIN, R. et ARON, M. 1927 Recherches sur l'évolution embryonnaire des îlots endocrines chez le poulet. C R. de la S. de Biol, 96.
- WEICHSELBAUM, A. und KYRLE, J 1909. Über das Verhalten der Langerhansschen Inseln des menschlichen Pankreas im fötalen und post-fötalen Leben. Arch. Mikro Anat Entw.-Gesch, 74.

EXPLANATION OF PLATE III

- Fig. 1. Section of pancreas in stage of 35 mm. in body length, to show three islands surrounding the same blood capillary, and to show several cell components of islands. *A* fuchsinophile island cell with indistinct granulation and notched nuclear membrane, *b* blood corpuscle, *c* blood capillary, *ca* centro-acinus cells, *i*₁ mass composed of primary island cells only, *i*₂ mass composed of a fuchsinophile (*A*) and another primary island cell, *i*₃ primary island cell constituting a cell mass together with two other cells (*D*) which are homogeneously fuchsinophile, and the nuclei in which show some abnormalities in staining reaction. $\times 1060$. Mallory's stain.
- Fig. 2. Section of pancreas in stage of 45 mm in body length, to illustrate mainly an island composed of various kinds of island cells. *if* fuchsinophile cell, *ip* primary island cells, *is* secondary island cells, *zd* degenerating zymogenous cell. $\times 1060$. Mallory's stain.
- Fig. 3. Section of pancreas in stage of 35 mm in body length, to show basement membrane of zymogenous tissue interrupted by centro acinus cells *c* blood capillary, *ca* centro-acinus cells, *d* preterminal duct. $\times 1060$ Mallory's stain

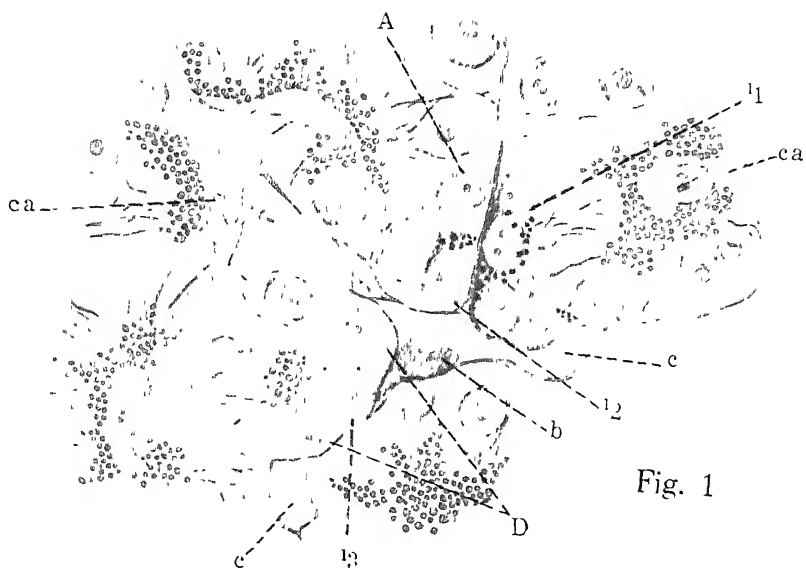


Fig. 1

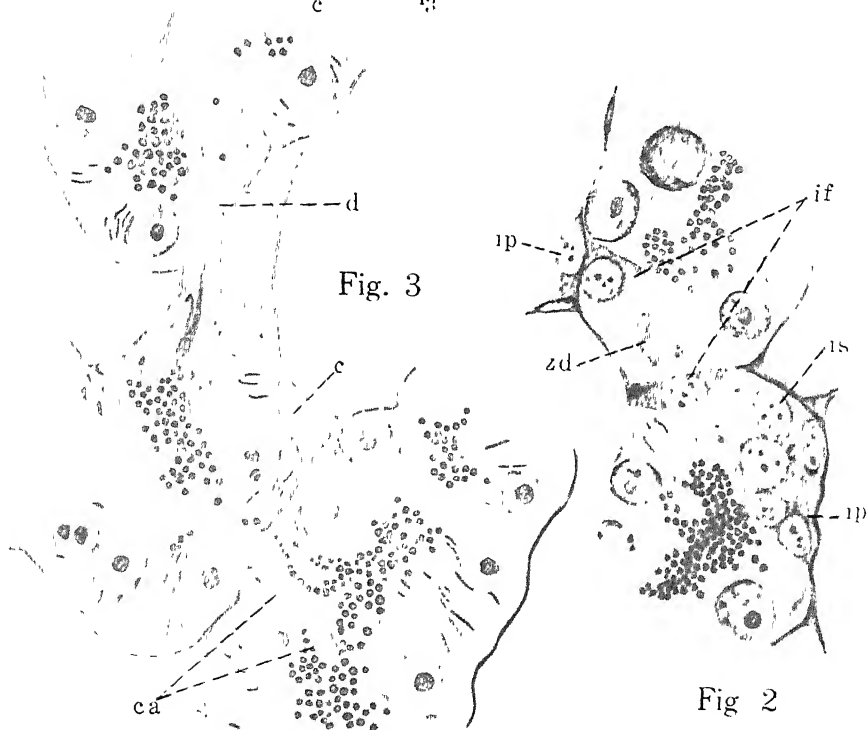


Fig. 3

Fig. 2

ON THE RELATION OF THE DAILY PERIOD TO THE SEXUAL MATURITY AND TO THE MOULTING OF *ZOSTEROPS PALPEBROSA JAPONICA*

By

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(With 28 text-figures)

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In Japan, from fairly ancient time, a method, which has been called "Yogai", has been widely practised by owners of pet domesticated birds such as

<i>Horeites cantans</i> (TEMMINCK and SCHLEGEL)	"Uguisu"
<i>Erithacus akahige</i> (TEMM.)	"Komadori"
<i>Zosterops palpebrosa japonica</i> (TEMM. and SCHL.)	"Mejiro"

"Yogai" is the method, by which the birds are brought to the light of a candle or of an electric or other light after sunset, to make them begin to sing their mating songs as early as possible in winter, even from the first day of January, i. e. before the spring, when wild birds usually begin to sing. Modern owners of pet birds generally subject the birds to the action of an electric light of from 50 to 60 watts for about from 3 to 4 hours after sunset, that is, in the winter in Japan, from about 4 to 7 or 8 o'clock in the afternoon. During the "Yogai", usually, the birds become less active than they are in the daytime, and sometimes, some of them completely cease their muscular exercise or fall asleep. All of them, however, begin to sing their mating songs after about a month from the beginning of the "Yogai", and even the birds which at first used to fall asleep, begin to sing on the same day and as well as those which were relatively active from the start.

It is usually said among owners of pet birds that in using this method a period of exposure of from 3 to 4 hours is the most effective and that any prolongation of this period is harmful to the birds. This fact has also been ascertained from my own experience in the past few years, though I can say nothing as to its exact cause at present.

There are several terms used to distinguish the varieties of songs of the birds. The songs sung during their breeding season are usually termed

“mating songs”, in the exactly same sense as used already in this paper. The cries which sound very sharp and harsh, namely “kiri-kiri-kiri” of *Lanius bucephalus* (TEMM. and SCHL.) from the top of trees on a very clear day of autumn, are called “alarm songs”, and do not express any desire for mating. Besides these, the terms “ordinary songs”, “flight songs”, etc. are used to distinguish the other kinds of songs of the birds.

Here I wish to emphasize the fact that the mating songs of the birds indicate their sexual maturity. In fact, a considerable acceleration of the period of mating songs by the “Yogai” directly implies the acceleration of sexual maturation. Thus it must be our question what factors of the “Yogai” cause the acceleration of sexual maturation?

If we compare all the factors of the daily period¹⁾ of spring, in which season the wild birds attain sexual maturity, with those of the “Yogai”, the real nature of the causal relation between the daily period and the sexual development may perhaps be explained. For the purpose of this study, the following, most easily approachable four factors were taken into consideration by myself:—

Rise of temperature

Prolongation of light period

Prolongation of feeding period

Prolongation of period of muscular exercise

Of these factors, the changes in the environment in spring and those in the environment caused by the “Yogai” are compared in what follows:

1) Temperature. Even though the birds are kept indoors there must be a difference between the temperature of cold winter and that of warm spring.

2) Light Period. The light period is longer in spring than in winter. In other words, in winter the sunlight period is much shortened, and in the “Yogai” this shortening of the sunlight period is compensated for by the electric light. The electric light, however, is the red light the wave length of which is limited so as to be above 400 λ . Thus, as far as the wave lengths are concerned, there must be a considerable difference between the sunlight and the electric light.

3) Feeding period. This period appears to me to be practically the same in spring and for the “Yogai”.

¹⁾ BISSONETTE, T. H. 1931. Studies on the sexual cycle in birds IV Experimental modification on the sexual cycle in males of the European starling (*Sturnus vulgaris*) by changes in the daily period of illumination and of muscular work Journ. Exp. Zool., Vol. 58, Pp 281-319.

4) Muscular exercise. The muscular exercise of the birds is somewhat less during the "Yogai" than in the daytime, some ceasing their movements and some falling asleep. But, as already stated, since the period of beginning the mating songs can be the same both in the case of the birds which are relatively active and those which fall asleep, the effect of muscular exercise upon sexual maturation can be thought to be very slight.

From the above generalization of the four factors, the feeding period may be expected to play a very important part during the "Yogai" as well as in spring. But this is merely a supposition. If some analytic experiments are made upon these various factors, separately or combined with others, then we may be able to answer the questions: Which single factor is the most important? Are all the factors necessary? This purpose was the real motive and interest which led the present writer to study one of the ecological problems suggested by the title of this paper, *Zosterops palpebrosa japonica* being selected as the material.

Zosterops palpebrosa japonica belongs to Zosteropidae, and is widely distributed over Honshiu, especially densely in the southern islands of Japan, including Shikoku and Kiushiu, and spreading over to the tropical islands. The breeding season of the wild birds ranges, in general, from the end of April to the end of July, and the moulting season from the beginning of September to the middle of October. In the localities, that are south-west from the middle region of Honshiu (Kanto) and are warm, there is no evidence that they migrate, but in the north-east region of Honshiu (Tôhoku), where the climate is rather cold in winter, they seem to migrate to a warm district during about from November to April. The migration records in Kurihara-Gun, Miyagi-Ken, which is about 30 miles north of Sendai, are shown in Table 1¹⁾.

TABLE 1

	First date of migration in spring	First date of migration in autumn
1931	April 3	September 28
1932	April 8	October 10
1933	March 31	September 26

¹⁾ These records are based on a private letter from my friend Mr. K. HAYAKAWA.

The wild birds feed on the honey of flowers, fruits, juice from the bark of some trees, and insects. The domesticated ones are usually fed on ground food containing 33% in weight of fish powder made from baked and dried freshwater fish.

Before entering on the subject of this paper, I wish to express my heartfelt thanks to Prof. E. NOMURA for his cordial guidance made during the progress of my work and for his kind revision of this manuscript. My thanks are also to offer to Prof. Y. YOSHII for his kind consent to my using his laboratory without any restrictions, to Assist. Prof. I. MOTOMURA for his kind advice, to Mr. S. SEKINE, Member of the Club of the Owners of Pet Birds in Sendai, for his kind assistance in feeding the birds, and to Mr. K. HAYAKAWA, a special owner of pet birds in Kurihara-Gun, Miyagi-Ken, for the kind information sent by him in relation to migration, etc.

In the course of my investigation three different sets of experiments were carried out, and in the present paper these are separately described under different headings.

I. RELATION BETWEEN THE DAILY PERIOD AND THE SEXUAL MATURATION

MATERIAL AND METHOD

The specimens of *Zosterops palpebrosa japonica* were captured with bird-lime in July, 1932, in the Chichibu Ranges, Saitama-Ken, which is the middle region of Kanto. The total number caught was 50. Of these 36 males were presumed to be of same age, because of their hatching invariably in May, 1932. Each young male bird was kept in a bamboo cage 10 inches in depth, 6 in breadth, and 7 in height. All the young were fed upon the ordinary ground food which contained 33% of fish powder. The food was renewed once every morning and left in the cage, so that the bird was able to feed at will. Care was taken that the composition of the food should not vary. None of them was sick until December 1. Already by this time, they had forgotten the fear which they had when they were caught, and were so domesticated that they returned to their own cages even after being allowed to fly out.

The present experiment was carried on from December 1. The laboratory used faced towards the south, and there was enough light to light up every part of the room. In the daytime, as the windows were kept open, the temperature within the room was practically the same as

that outside, but at night, as the windows were closed in order to protect the birds from possible enemies, the temperature of the room was higher by 2° – 3° C than that out-of-doors. The electric light employed in the "Yogai" was a tungsten lamp of a power of 100 watts, and it was lighted from 4 o'clock to 8 in the afternoon at a distance of 3 meters from the bird-cages. In order to make the illumination uniform throughout, the positions of the cages were altered as often as possible.

Of the 36 birds, 26 were exposed to the "Yogai", the other 10 remaining in darkness as the control. All lights were shut off from the control birds by covering their cages with black cloth at 4 o'clock in the afternoon.

EXPERIMENTAL RESULTS AND REMARKS.

On December 20, just 20 days after the beginning of the "Yogai", the first mating songs were heard from one of the "Yogai" group. Since then, the number of those singing mating songs gradually increased, and on January 5, all the specimens had begun to sing their mating songs. Furthermore, combats between those of the same sex became violent, and they seemed to behave in the manner is observable during the regular breeding season.

In the "Yogai" specimens, internally, the testes increased in size with the lapse of time until the birds began to sing. At the very beginning of the experiment on December 1, the seminiferous tubules were slender, and the spermatogenetic stages were practically unobservable (Fig. 1). Then, with the lapse of time the tubules increased in diameter, the spermatogenetic figures having become visible (Fig. 2). Finally, when the birds began to sing their mating songs, the diameter of the tubules reached its maximum and the formation of spermatozoa was accurately observed (Fig. 3).

In the case of the control specimens, however, the development of the testes, even on January 5, remained in the state in which it was at the very beginning of the "Yogai", and the formation of spermatozoa was only noticed later in the testes taken from a specimen on May 20, just at the outset of the breeding season of the wild birds.

From the above results of experiment, it may be definitely said that spermatogenesis is accelerated by the "Yogai", in support of the view of BISSETTE, who worked on *Sturnus vulgaris*¹⁾.

¹⁾BISSETTE, T. H. 1931 *loc. cit.*

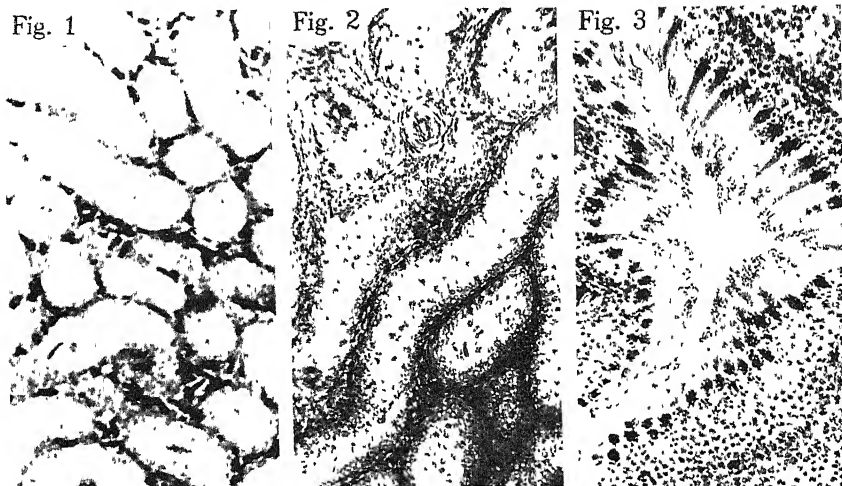


Fig. 1 Section of testis taken [from a specimen on December 1, at the very beginning of the "Yogai". $\times 200$

Fig. 2. Section of testis taken at random from a specimen on December 15, 15 days after the beginning of the "Yogai". $\times 200$. The diameter of the seminiferous tubule is about 1.5 times that shown in Fig. 1

Fig. 3 Section of testis taken at random from a specimen, which began to sing mating songs, on January 5, 36 days after the beginning of the "Yogai" $\times 200$

Since I was much interested in knowing how many times the sexual maturity could be repeated by the "Yogai", in order to begin my work along this line, the "Yogai" method was discontinued on April 1, the daily period thus returning to the normal conditions.

It is very interesting to me to state here that on April 14, just two weeks after the "Yogai" had ceased, a number of feathers began unexpectedly to fall off, and a month later new plumage had completely grown. I will discuss this unexpected phenomenon in the next section.

The birds, with their plumage renewed, were again exposed to the action of "Yogai" from September 1. Since the procedure of the "Yogai" and its results, including the stoppage of it on October 30 and the moulting which began on November 15, are exactly the same as in the first experiment, the details need not be duplicated here.

The third "Yogai" was begun from December 1, before finishing the moulting, and it resulted as on the two previous occasions.

As just shown, therefore the sexual maturity of *Zosterops palpebrosa japonica* can be repeated at least three times a year by exposure to the action of the "Yogai", and from this fact it is clearly confirmed that

there is an intimate relation between the daily period and the development of sexual maturity.

II RELATION BETWEEN THE DAILY PERIOD AND THE MOULTING

In the preceding section, attention was called to the following points:

1) The regular moulting season of *Zosterops palpebrosa japonica* lasts for about a month ranging from the earlier part of September to that of October.

2) The specimens of the bird under discussion, which attained sexual maturity in the course of the "Yogai" on January 5, unexpectedly began moulting after the "Yogai" had stopped on April 1, and also in November the second moulting.

From these facts, it may be considered that, although the moulting season of the wild specimens begins ordinarily at the beginning of September, it is also compelled to begin as early as in April by shortening the daily period, that is, strictly speaking, by stopping the "Yogai". Even though it seems to be true that a definite relation may exist between the shortening of the daily period and the moulting, as is probably the case between the prolongation of the daily period and the acceleration of sexual maturity, yet the possible factors which may cause the moulting in natural conditions ought to be investigated. On considering first the natural environment in which the wild birds undergo moulting from the beginning of September, the following factors may be distinguished:

Climatic change

Relation with sexual maturity

Daily period

Light Period

Feeding period

1) With regard to the climatic change, at the beginning of September the hot weather of August is just becoming moderate and changing towards cold. Yet the periods of moulting, which happened to occur as the result of stopping the "Yogai", were April and November. In the former the weather changes to warm and moderate, while in the latter it changes towards the cold winter. From these contradictions, it appears to me that climatic conditions, especially temperature, have a very slight connection with the moulting.

2) Concerning the relation with the sexual maturity, the testes of the wild specimens were entirely dedifferentiated at the beginning of September

(Fig. 4), but in the testes of the specimens, which already began the moulting in either April or November, respectively, not only were no signs of dedifferentiation observable, but also the formation of the spermatozoa was still continuing (Fig. 5).

Fig. 4

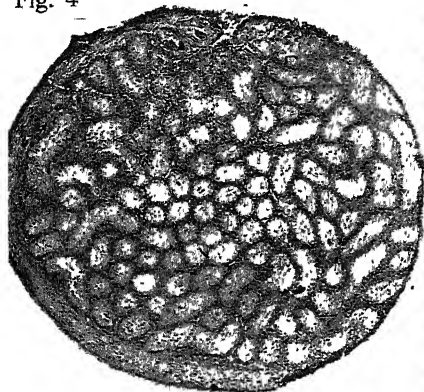


Fig. 4. Section of testis from a wild specimen caught in the upper part of September. $\times 80$.

Fig. 5

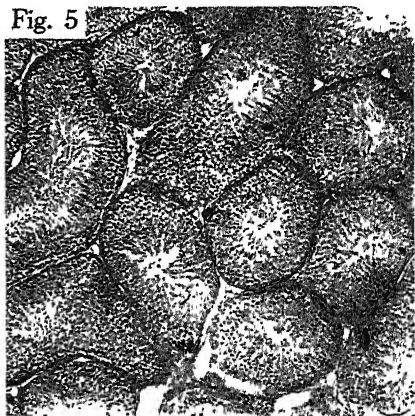


Fig. 5. Section of testis from a specimen which began the moulting in April after the "Yogai" had ceased. $\times 80$.

If the moulting were to occur constantly after finishing the period of sexual development, there could never be such a difference between the structure of the testes shown in the regular moulting season, after the regular breeding season, and that shown in the moulting period after the arrival at sexual maturity caused by the "Yogai". Furthermore, in the natural condition, the young birds, which hatch in May and which have never passed through the first breeding season, invariably undergo moulting in September of the same year. This fact seems to confirm most strongly the view that there is no direct relation between the full development of the testes and the moulting.

3) The light period in September is shorter than that in the breeding season, which usually extends from the middle of May to the latter part of July. Consequently, the feeding period is also shorter in the moulting season than in the breeding season. These differences in the periods suggested to me the view that a shortening of both the light and feeding periods might play an important part in causing the moulting. My present experiment therefore was made as follows.

MATERIAL AND METHOD

The specimens used were 36 in number, and were captured in the Chichibu Ranges in June, 1933. All the birds were hatched in May, 1933. Consequently their first moulting was to occur in September, 1933. On July 14, these birds were separated into three groups, viz. A, B, and C, each group consisting of 12. The light and feeding periods of the groups were controlled, respectively, as shown in Table 2.

TABLE 2

	Light period	Feeding period
Group A	10 hours	10 hours
Group B	15 hours	10 hours
Group C	15 hours	15 hours

All the groups were provided equally with the ground food at 6 o'clock, every morning. Of these groups, Group A was moved to a dark room, where the "Yoga" was being carried out, every day, at 4 o'clock in the afternoon, until 6 o'clock next morning, from the usual room, where this group was placed together with other groups in the daytime and where the daylight shone in. Thus the feeding period as well as the light period of Group A was confined to 10 hours a day. Group B remained all day in the usual room, but the food was taken away every day at 4 o'clock in the afternoon; the feeding period of this group thus being 10 hours a day and the light period nearly 15 hours. Group C was placed all day in the usual room without removal of the food, so that both the light and feeding periods of this group were presumed to be nearly 15 hours a day.

I would also have made another group, in which the light period was 10 hours a day and the feeding period 15 hours. But this group was not actually constituted owing to the difficulty that the birds never feed in the dark.

EXPERIMENTAL RESULTS AND REMARKS.

The experiment was started on July 14. On July 24, since it was noticed that a pair of feathers had fallen off from two birds in Group A, I then continued an exact count of the number of falling feathers

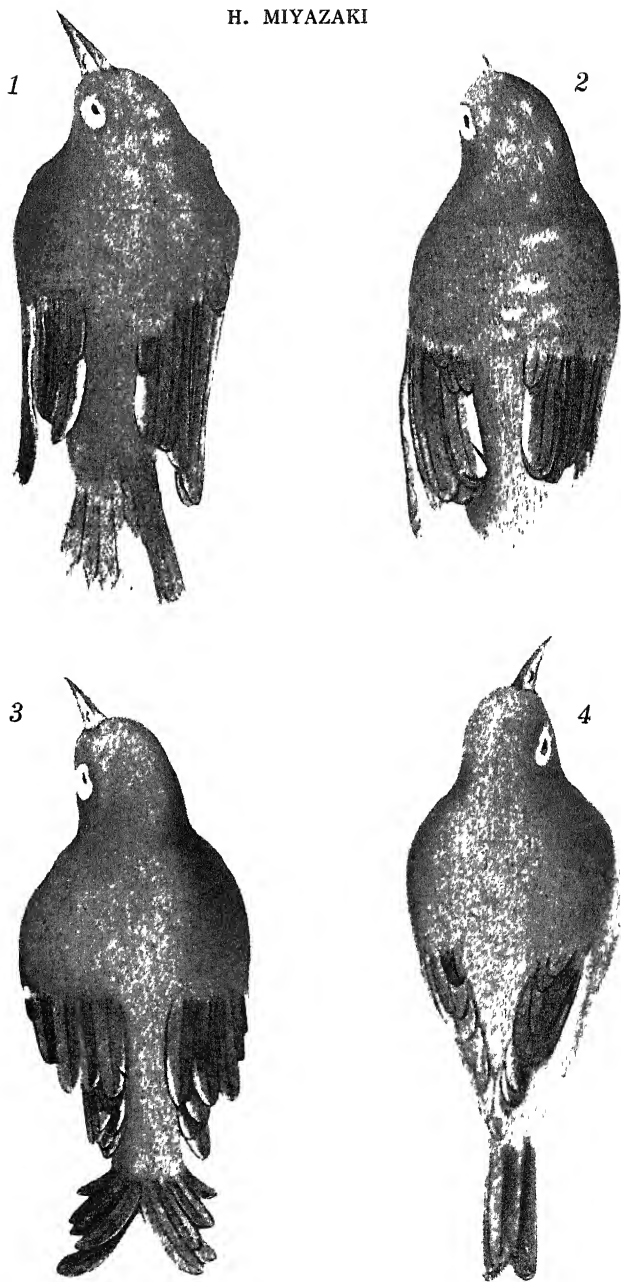


Fig 6 Four stages of the moulting of *Zosterops palpebrosa japonica*, in which the moulting had begun on July 24. 1 moulting at about its beginning on about July 27, 2 moulting fairly advanced with the rectrices fallen off on about August 7, 3 new down feathers and contour feathers growing in the latter part of August, 4 moulting completed at the end of August Natural size.

beginning from July 25. In the course of time, in Group A, the specimens which had begun the moulting increased in number, and on about August 10 a considerable number of fallen feathers were found. In both Group B and Group C, however, any signs of moulting were not recognizable until August 22, on which date only one feather from one bird in Group B was recorded. On September 7, after 50 days from the beginning of this experiment, the moulting became marked even in these groups.

At the end of August, the moulting of Group A had already passed and the new plumage seemed to have been completed (Fig. 6), but Groups B and C, with similar slowness, finished moulting nearly 50 days later than Group A. The relation between the date and the average number of feathers fallen off is shown in Fig. 7.

1) A comparison of the data from Group A and from Group B confirms the fact that the acceleration of the moulting period in Group A is due to the light period in the case of Group A being shorter than that in the case of Group B.

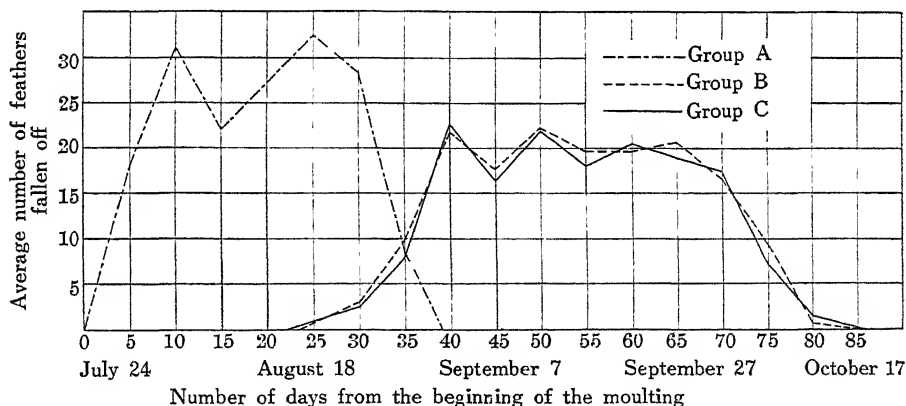


Fig. 7. A graph showing the relation between the number of feathers fallen from one bird (average from 12 birds of each respective group, every 5 days), and the moulting periods shown by the different groups of the birds.

2) A comparison of the data from Group A and from Group C confirms the fact that the acceleration of the moulting period of Group A is due to the light period in the case of Group A being shorter than that in the case of Group C.

3) A comparison of the data from Group B and from Group C confirms the fact that the similar delay of the moulting period in both groups is caused without regard to the different lengths of the feeding period.

As we see in the above comparisons, the shortening of the light period causes an acceleration of the moulting period, but the length of the feeding period appears to have no differential effect on the moulting period. It is an interesting conclusion that the moulting period is accelerated by a shortening of light period, while the period of sexual development to maturity is accelerated by a prolongation of the daily period.

It ought to be explained here Why the feeding period was confined to 10 hours a day? Before the present experiment was undertaken, I had previously compared the nutritional difference between the birds of the 10-hour feeding period and those of the 9-hour period. The latter birds became very weakened on the third day and finally died, while the former ones were invariably healthy, although sometimes evidently hungry. In the present experiment therefore the 10-hour feeding period was preferred to place the birds in the maximum condition of under-nourishment without causing death. Moreover, the light period and the period of muscular exercise are so closely related, that at present these factors cannot be perfectly distinguished by experiment, because in the dark the birds make no movements, and I was unable to find any adequate method of making them exercise without frightening them. Therefore, not only in this section but also in the next one, "light period" ought to be understood as "light period as well as period of muscular exercise".

III. RELATIONS BETWEEN THE SEXUAL MATURATION AND VARIOUS FACTORS IN THE DAILY PERIOD

It has already been mentioned that the sexual maturation of *Zosterops palpebrosa japonica* is caused by a prolongation of the daily period. The present experiment was undertaken in order to determine how each factor of the daily period is related to the sexual development.

Of all the factors included in the daily period, the following three were selected as the most important ones:

- Light period
- Feeding period
- Temperature

MATERIAL AND METHOD

All the specimens used in this experiment were captured at the same time as those used in the preceding experiment and from the same locality and were birds invariably hatched in May, 1933. The rooms used were

the usual room, the dark room, and the green house. As to the usual room and the dark room, the conditions have already been described. The green house was lighted up to the same extent as the usual room. The average temperature of these three different rooms, during from December 1, 1933, to January 28, 1934, is shown in Table 3.

The dark room was used only for the "Yogai" from 4 o'clock to 8, afternoon. The slightly higher temperature in the dark room than in the usual room was due to the heat from the electric light.

TABLE 3

	Average of maximum temperature	Average temperature	Average of minimum temperature
Green house	24°C	15.5°C	9°C
Usual room	7°C	3.1°C	-4°C
Dark room	—	5.3°C	—

106 specimens consisting of 80 males and 26 females were divided into 6 groups, viz. A, B, C, D, E, and F, the feeding and light periods and the temperature of the respective group being limited as shown in Table 4.

TABLE 4

	No. of birds		Feeding period in hours	Light period and period of muscular exercise in hours	Place
	Male	Female			Respective temperature of the places shown in Table 3.
Group A	15	6	14	14	Usual room Dark room, from 4-8 PM.
Group B	15	6	10	14	Usual room Dark room, from 4-8 PM.
Group C	10	4	10	10	Usual room
Group D	10	4	14	14	Green house
Group E	15	3	10	10	Green house
Group F	10	3	10	10	Outside, during daytime Usual room, during night

Among the groups the experiment was started on Groups A, B, C, E, and F from December 1, 1933, but Group D was separated from Group C on December 10, thus the experiment with this group was started 10 days later than the start of the other groups.

The daily period in this season was presumed to be nearly 10 hours.

For the purpose of the "Yogai", Groups A and B were moved, every day, from the usual room to the dark room at 4 o'clock in the afternoon, the food having been taken away from Group B, and as soon as the "Yogai" finished at 8 o'clock these groups returned again to the usual room. The "Yogai" of Group D was carried on in the green house from 4 o'clock to 8, afternoon, and, while the electric lamp was lighted the cages of Group E were completely covered with dark cloth.

Whether or not the birds had attained sexual maturity was determined by the following criteria:

Size of the testes and ovaries

Histological differentiation of the testes and ovaries

The singing of the mating songs must be here mentioned. Actually, on December 4, I could hear a mating song from one bird of Group E, which was placed in the green house, and next day also from another one of the same group. The singing continued for about 3 days, but after that was never heard again. The histological examination of the gonads proved that those were still far from maturity. I thought at first that the birds might sing their mating songs even before entering on sexual maturity, if they were placed in a suitable, comfortable, and pleasant condition. I now, however, doubt whether those songs were real mating songs, so that I have decided to omit this criterion, because the two criteria mentioned above are quite sufficiently effective to determine the degree of the development of sexual maturity, though to do this requires a difficult technique.

OBSERVATIONS

Notes on December 1, 1933.

At the very beginning of this experiment, both the testes and ovaries are very small and are, as expected, in an early stage of histogenesis. The spermatogonia are distributed in the slender seminiferous tubules, and in the intertubular space the pigment and interstitial cells¹⁾²⁾³⁾ are richly found (Fig. 8). The growth of some of the oögonia had already begun (Fig. 9).

¹⁾ NONIDEZ, J. F. 1924. Studies on the gonad of the fowl. IV The intertubular tissues of the testis in normal and hen-feathered cocks. *Amer. Journ. Anat.*, Vol. 34, Pp. 359-392.

²⁾ RASMUSSEN, A. T. 1928. Interstitial cells of the testis. *Special Cytology*. New York.

³⁾ BISSONETTE, T. H. 1931. *loc. cit.*

Fig. 8

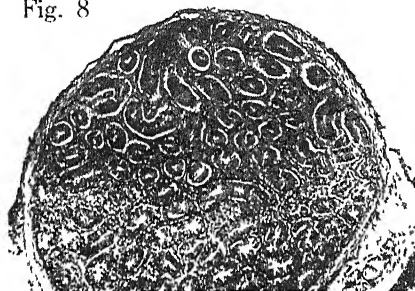


Fig. 9

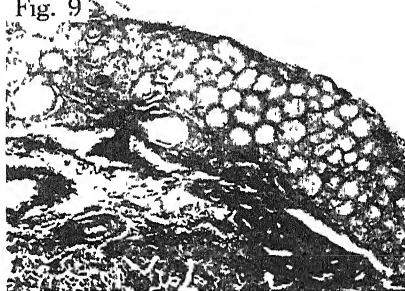


Fig. 8 Section of testis taken from a specimen on December 1, 1933, at the beginning of this experiment. $\times 80$

Fig. 9. Section of ovary taken from a specimen on December 1, 1933, at the beginning of this experiment. $\times 80$

Notes on December 20, 1933.

Groups A and B. The testes and ovaries are pretty well developed and almost 3 times as large in diameter as those of Groups C, E, and F. The formation of spermatocytes and the diminution of interstitial and pigment cells are observed (Figs. 10 and 12). The oögonia are much enlarged (Figs. 11 and 13).

Groups C, E, and F. The size and the state of histogenesis of the gonads remain practically the same as those observed on December 1

Notes on December 30, 1933.

Group D. Since the experiment of this group was begun 10 days later, December 30 for this group just corresponds to December 20 for the other groups. The degree of development of the gonads is practically the same as those of Groups A and B (Figs. 14 and 15).

Notes on January 18 (Groups A, B, C, E, and F) and on January 28 (Group D), 1934.

The size of the gonads of Groups A, B, and D are very much enlarged, and measures almost 8 times in diameter that of the other groups, which remained nearly unchanged (Fig. 16).

Groups A, B, and D. The groups are those exposed to the "Yogai". In the testes the formation of spermatozoa is observed (Figs. 17, 19, and 21). In the ovaries the yolk contents of oögonia are considerably increased, and the largest one may already be called the primary oöcyte (Figs. 18, 20, and 22).

Groups C, E, and F. The state of the histogenesis of the gonads is practically the same as that of December 1, 1933 (Figs. 23-28).

Fig. 10

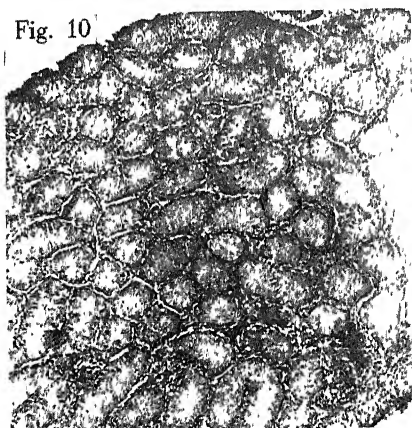


Fig. 11

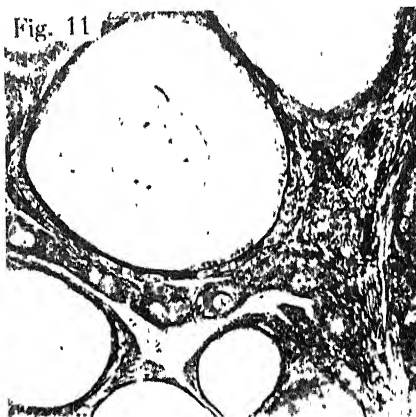


Fig. 12

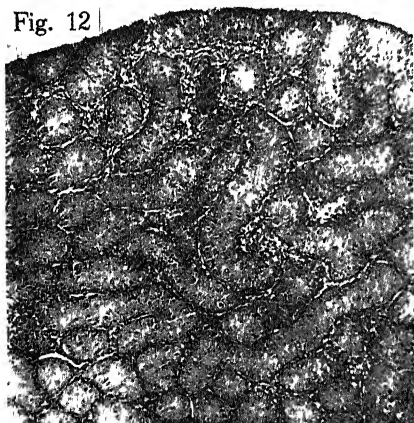


Fig. 13

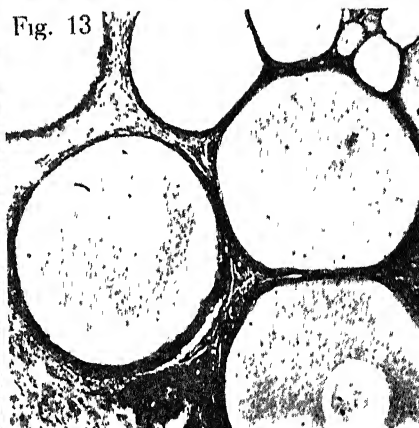


Fig. 14

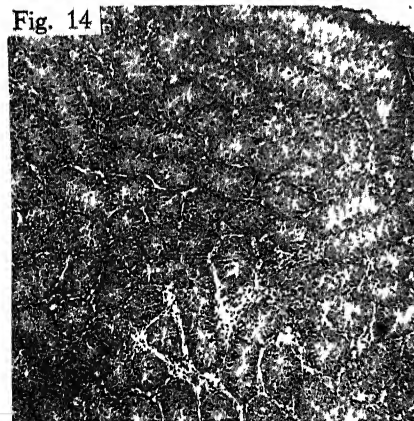


Fig. 15

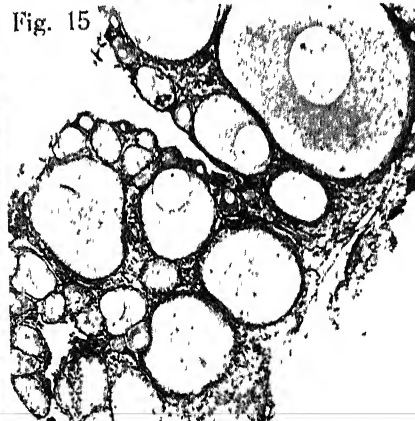


Fig. 10. Section of testis from a specimen of Group A on December 20, 1933, just 20 days after the beginning of this experiment $\times 80$

Fig. 11. Section of ovary. Group A December 20, 1933. $\times 80$

Fig. 12. Section of testis. Group B December 20, 1933 $\times 80$

Fig. 13. Section of ovary. Group B. December 20, 1933 $\times 80$.

Fig. 14. Section of testis. Group D December 30, 1933 $\times 80$

Fig. 15. Section of ovary. Group D. December 30, 1933, $\times 80$

Notes on March 4, 1934.

At the close of this experiment, the size of the gonads of Groups C, E, and F, respectively, remained apparently in the same state as those taken on January 18, while in the case of the birds of Groups A, B, and D, the ovulation was already recognized. Moreover, at the close of this experiment, a comparison of nutritional conditions between Group A and Group B was made. In both groups no apparent difference was found in quantity of fat, and Group B was rather heavier than Group A in weight of the respective 5 birds.

EXPERIMENTAL RESULTS AND REMARKS

1) Comparison of Group C and Group F. The approximation of the light period, feeding period, and of the temperature brings practically



Fig. 16. Testes (upper) and ovaries (lower), to show their real size

A, B, C, D, and E represent respectively Groups A, B, C, D, and E.

A, B, C, and E taken on January 18, and D on January 28, 1934.

Fig. 17. Section of testis taken from a specimen of Group A on January 18, 1934, just 49 days after the beginning of the experiment $\times 80$

Fig. 18. Section of ovary. Group A. January 18, 1934 $\times 80$.

Fig. 19. Section of testis. Group B. January 18, 1934 $\times 80$.

Fig. 20. Section of ovary. Group B. January 18, 1934 $\times 80$

Fig. 21. Section of testis. Group D. January 28, 1934. $\times 80$

Fig. 22. Section of ovary. Group D. January 28, 1934. $\times 80$.

Fig. 23. Section of testis. Group C. January 18, 1934. $\times 80$

Fig. 24. Section of ovary. Group C January 18, 1934 $\times 80$

Fig. 25. Section of testis. Group E. January 18, 1934 $\times 80$

Fig. 26. Section of ovary. Group E. January 18, 1934. $\times 80$

Fig. 27. Section of testis. Group F. January 18, 1934 $\times 80$

Fig. 28. Section of ovary. Group F. January 18, 1934. $\times 80$

Fig. 17



Fig. 18

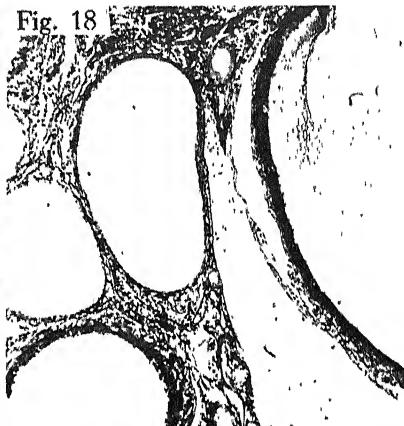


Fig. 19



Fig. 20

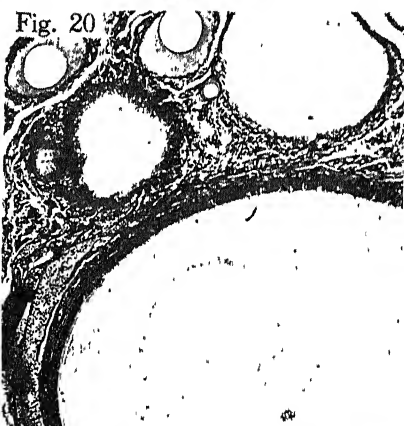


Fig. 21



Fig. 22

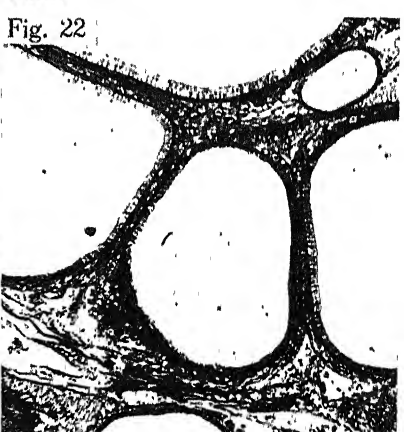


Fig. 23

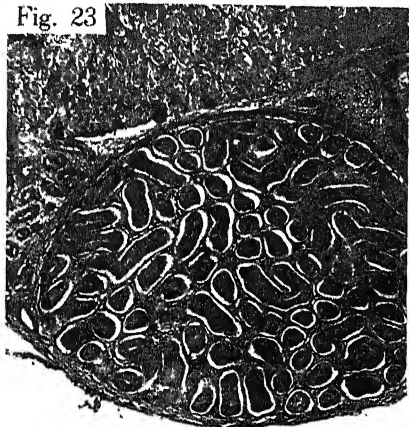


Fig. 24

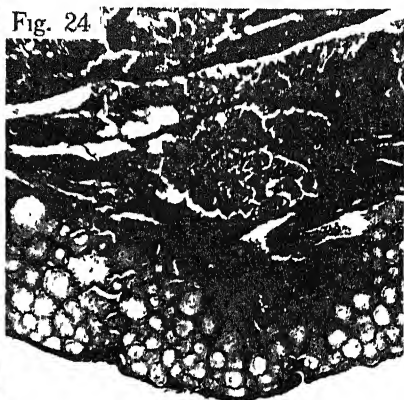


Fig. 25

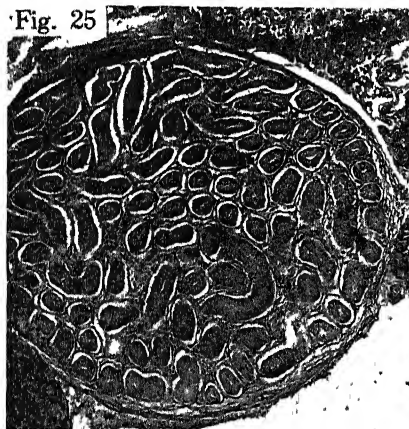


Fig. 26

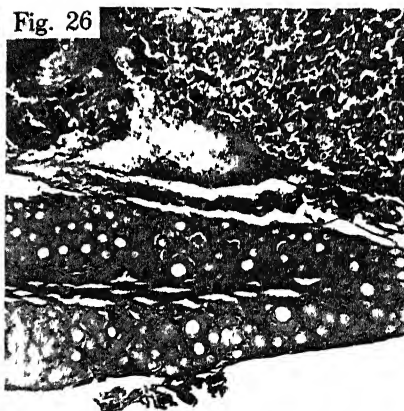


Fig. 27

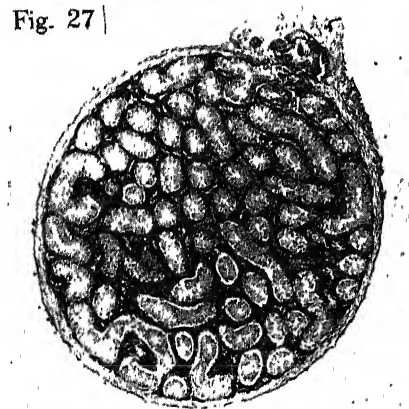
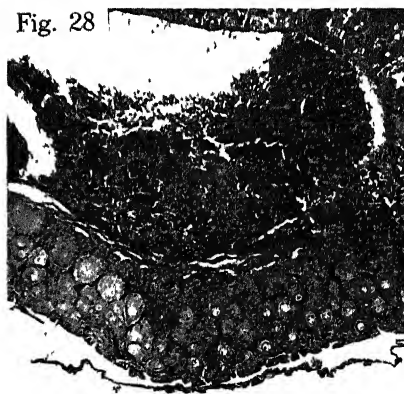


Fig. 28



similar results in connection with the sexual maturation.

2) Comparison of Group C or F and Group D. The prolongation of the light and feeding periods as well as the high temperature of the green house appear to accelerate the sexual maturation of Group D.

3) Comparison of Group A or D and Group C or E or F. The prolongation of both the light and feeding periods accelerates the sexual maturation, without regard to the differential temperature. This results is the same as that obtained in the first experiment, viz. that the acceleration of the sexual maturation is due to a prolongation of the daily period.

4) Comparison of Group E and Group C or F. The differential temperature has practically no influence to the sexual maturation.

5) Comparison of Group A and Group D. While the lengths of the light period and of feeding period are constant, the experimental results are practically the same, without regard to the influence of the differential temperature.

6) Comparison of Group B and Group E. While the feeding period is constant the sexual maturation is accelerated by the prolongation of the light period, without regard to the high temperature. Thus the temperature may be omitted when we are concerned only with the important factors causing the acceleration of the sexual maturation.

7) Comparison of Group B and Group C or F. While the feeding period is the same, the prolongation of the light period undoubtedly accelerates the sexual maturation.

8) Comparison of Group B and Group D. The sexual maturation is accurately accelerated by the prolongation of the light period, without regard to the differential length of the feeding period or to the differential temperature. Thus not only the temperature but also the feeding period diminishes in importance.

9) Comparison of Group A and Group B. The differential lengths of the feeding period do not influence differentially the degree of the sexual maturation, while the length of the light period is constant. Thus the feeding period may also be omitted from the important factors.

At first I was intending to make one more group in which the light period lasted 10 hours, and the feeding period 15 hours, a day, but this group was not actually made owing to the difficulty stated in the preceding section. Fortunately, however, this defect was counterbalanced by a comparison of Group B with Groups A and C.

From the above comparisons, it is confirmed that the light period as well as the period of muscular exercise plays the most important rôle in

causing the acceleration of the sexual maturation, while any effect of the feeding period and of temperature is quite unappreciable.

Whenever the birds were moved to the dark room for exposure to "Yogai", they used to cease muscular exercise, although they were exposed to the electric light, and many of them looked very sleepy, placing the head behind the perch or the food dish and partly closing the eyes. Those of Group A sometimes ate the food, but there were no movements visible as seen in the daytime. Those of Group B, from which the food had been taken away, used to make no movements, even when one's hand approached them. Thus, the effects of the prolongation of the period of muscular exercise appear to me to be much smaller than those of the prolongation of the light period.

SUMMARY

1) Three experiments were carried out to determine the relations of daily period to the sexual maturation and to the moulting of *Zosterops palpebrosa japonica*.

2) In order to lengthen the daily period, an electric light of 100 watts was used.

3) The sexual maturation is accelerated by a prolongation of the daily period.

4) The sexual maturity can be repeated at least three times a year by means of the prolongation of daily period.

5) Among the factors of the daily period, the light period is most important in causing the acceleration of the sexual maturation, while feeding period, temperature, and muscular exercise are less important.

6) A shortening of the light period causes an acceleration of the moulting period, but differential length of feeding period has no differential effect on the moulting period.

RELATION BETWEEN THE WEIGHT, VOLUME, AND LINEAR DIMENSIONS IN *MERETRIX MERETRIX* (L.)

By

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(With one text-figure)

(Received July 13, 1934)

The formulae expressing the relations between the depth (D), height (H), weight (W), and length (L) of the molluscan shells, which have been given by NOMURA¹⁾, and which have been further confirmed by HAMAI³⁾, are, respectively, as follows:

$$D = a_1 L^{b_1} \text{-----} (1)$$

$$H = a_2 L^{b_2} \text{-----} (2)$$

$$W = a_3 L^{b_3} \text{-----} (3)$$

where a and b are constants.

From the formulae in (1), (2), and (3), the following relations may be obtained, in which K , α , β , and γ are constants:

$$\left. \begin{aligned} W &= K_1 D^\alpha H L \\ &= K_2 D H^\beta L \\ &= K_3 D H L^\gamma \end{aligned} \right\} \text{-----} (4)$$

NOMURA⁴⁾ has shown that the relation

$$\alpha = \beta = \gamma \text{-----} (5)^5)$$

exists with regard to formulae in (4) in the case of the bivalves, *Tapes philippinarum* and *Meretrix (Cytherea) meretrix*.

¹⁾ NOMURA, E. 1926. An Application of $a=kb^x$ in Expressing the Growth Relation in the Freshwater Bivalves, *Sphaerium heterodon* PUS. Sci. Repts. Tôhoku Imp. Univ., Biol., Vol. II, Pp. 57-62.

²⁾ NOMURA, E. 1926. Further Studies on the Applicability of $a=kb^x$ in Expressing the Growth Relations in Molluscan Shells. Sci. Repts. Tôhoku Imp. Univ., Biol., Vol. II, Pp. 63-84.

³⁾ HAMAI, I. 1934. On the Local Variation in the Shells of *Meretrix meretrix* (L.), with Special Reference to Growth of Organism. Sci. Repts. Tôhoku Imp. Univ., Biol., Vol. IX, Pp. 131-158.

⁴⁾ NOMURA, E. 1928. On the Relation between Weight and Dimensions in the Bivalves, *Tapes philippinarum* and *Cytherea meretrix*. Sci. Repts. Tôhoku Imp. Univ., Biol., Vol. III, Pp. 113-124.

⁵⁾ $\alpha = \frac{b_3 - b_1 - 1}{b_1}$, $\beta = \frac{b_3 - b_1 - 1}{b_2}$, $\gamma = b_3 - b_2 - b_1$.

The present investigation has been undertaken to re-examine the applicability of formulae in (4) and (5), then to test whether or not a similar relation can also exist between the volume and the linear dimensions, and finally, to ascertain the weight-volume relation from the weight-length and volume-length relations. This investigation has been made at the suggestion of Prof. E. NOMURA to whom I wish to express my hearty thanks for his kind guidance during the course of this work.

WEIGHT-RELATION WITH THREE LINEAR DIMENSIONS

For the purpose of the re-examination of the relations, 12 tables (Tables 4-13, 16, and 17), out of 15 given in my previous paper³⁾ are referred to, the remaining 3 (Tables 3, 14, and 15) being omitted because of the insufficient number of individual specimens available.

As shown together in Table 1, the calculated values of the respective exponents, α , β , and γ in formulae in (4), indicate that relation (5) holds good approximately when we are concerned only with one habitat, but the value of the exponents differs according to the difference in locality.

TABLE 1.

Place	α	β	γ	Average
Saizyô-Mati	0.73	0.72	0.73	0.73
Turesima-Mati	0.46	0.39	0.40	0.42
Takamatu-Mati	0.89	0.87	0.89	0.88
Ryôkai-Mura	0.79	0.78	0.79	0.79
Yatuya-Mati	0.67	0.64	0.67	0.66
Yatusiro-Mati	0.59	0.51	0.55	0.55
Tokusima	0.67	0.64	0.68	0.66
Ôzî-Mura	0.66	0.64	0.67	0.66
Kawagoe-Mura	0.51	0.46	0.51	0.49
Kankawa	0.72	0.68	0.71	0.70
Watanoha-Mati	0.98	0.98	0.98	0.98
Kusatu-Mati	0.78	0.73	0.76	0.76

VOLUME-RELATION WITH THREE LINEAR DIMENSIONS

The volumetric determination was carried out by using the volumetric apparatus shown in Fig. 1. This apparatus is composed of a funnel-tube

³⁾ HAMAI, I. 1934 *loc. cit.*

A with a narrow side tube B, a burette D, and a rubber tube C connecting A and D. The funnel-tube was large enough to contain a clam of considerable size. In order to keep the water level constant when the apparatus contains water, a circular line was marked round the side tube, the calibre of which was as wide as that of the burette. The latter was easily movable vertically. The wall of the rubber tube was thick enough to prevent any swelling due to the pressure of water: such swelling would make the measurements incorrect.

At first, the apparatus was filled with tap water to the level of the circular line on the side tube, and the scale on the burette was then read. A clam was then gently introduced into the funnel-tube, the burette was moved downwards until the water level again reached the circular line on the side tube, and a reading of the burette was again taken. The difference in the two readings of the burette was taken as the volume of the clam. The volume taken is the total displacement of each perfectly closed clam and is calculated accurately to one decimal place in cc.

Since the volume-length relation can also be formulated similarly as in the case of the weight-length relation (3),

$$V = a_1 L^{b_1} \quad (6)$$

where V denotes volume. In the real value of a_1 and of b_1 , the formulae relating to the specimens collected from the 3 different places (Tables 4, 5, and 6) are as follows:

$$\text{Saizyô-Mati} \quad V = 0.232L^{2.77}$$

$$\text{Turesima-Mati} \quad V = 0.179L^{2.95}$$

$$\text{Takamatu-Mati} \quad V = 0.186L^{2.82}$$

To express the relations between the volume and the three linear dimensions D , H , and L , exactly similar formulae those in (4) also applied, being derived from (1), (2), and (6), hence

$$\left. \begin{aligned} V &= K_1 D^{\sigma} H L \\ &= K_2 D H^{\beta} L \\ &= K_3 D H L^{\gamma} \end{aligned} \right\} \quad (7)$$

The calculated results of σ , β , and γ are shown together in Table 2, and this table shows that the relation found in the case of the weight is also applicable to that of the volume.

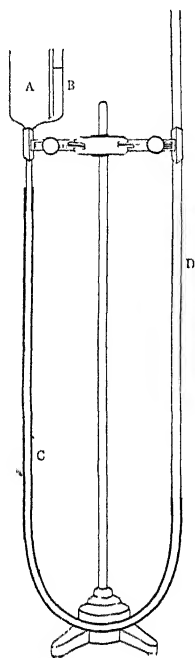


Fig. 1. Volumetric apparatus.

TABLE 2.

Place	α	β	γ	Average
Saizyô-Mati	0.82	0.81	0.82	0.82
Turesima-Mati	0.87	0.85	0.85	0.86
Takamatu-Mati	0.95	0.94	0.95	0.95

WEIGHT-VOLUME RELATION

From (3) and (6), the weight-volume relation

$$W = a_5 V^{b_5} \quad (8)^{a)}$$

may be obtained. As to the above three places, where the real values of a and b are, respectively, recapitulated in Table 3, the following are the actual relations found, namely

TABLE 3

Place	Volume-length relation		Weight-length relation	
	a_4	b_4	a_3	b_3
Saizyô-Mati	0.232	2.77	0.188	2.68
Turesima-Mati	0.179	2.95	0.227	2.50
Takamatu-Mati	0.186	2.82	0.201	2.76

$$\left. \begin{array}{ll} \text{Saizyô-Mati} & W = 0.776 V^{0.07} \\ \text{Turesima-Mati} & W = 0.980 V^{0.85} \\ \text{Takamatu-Mati} & W = 1.045 V^{0.08} \end{array} \right\} \quad (9)$$

As seen in the formulae of (9), the values of the exponent, accordingly, differ with the locality, and the weight-volume relation is, generally, not linear.

SUMMARY

1. In the shells of *Meretrix meretrix*, the following relations are approximately true:

$$\begin{aligned} U &= K_1 D^s H L \\ &= K_2 D H^s L \\ &= K_3 D H L^s \end{aligned}$$

$$^a) a_5 = \frac{a_8}{a_4^{b_5}} \text{ and } b_5 = \frac{b_3}{b_4}.$$

where U is weight or volume, D , H , and L , respectively, the depth, height, and length of the clam-shell, and K and ϵ are constants.

2. The value of ϵ differs according to the locality.

3. The value of the constant b varies according to the locality and is expressed by the formula, $W=aV^b$, where W and V indicate, respectively, weight and volume, a being another constant.

4. The differential value of the constants ϵ and b respectively may reveal that the shell development varies according to the environmental conditions.

TABLES AS THE BASES OF CALCULATION

TABLE 4. Saizyô-Mati, Ehime-Ken.

Collected on Nov. 20, 1932.

Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Volume in cc.	Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Volume in cc.
3.10	1.49	2.15	3.9	5.4	4.04	2.03	3.37	8.4	11.5
3.16	1.51	2.66	3.8	5.1	4.04	2.05	3.37	7.3	11.3
3.32	1.62	2.84	5.1	6.6	4.05	2.04	3.34	7.1	11.1
3.40	1.65	2.81	4.0	6.3	4.05	2.05	3.37	7.8	11.5
3.41	1.73	2.88	5.8	7.3	4.05	1.95	3.24	7.2	10.6
3.61	1.76	3.04	6.1	8.0	4.06	2.08	3.33	8.2	11.4
3.64	1.80	3.08	5.0	8.5	4.08	2.03	3.29	7.8	11.7
3.65	1.79	3.03	6.1	8.7	4.09	2.05	3.38	8.9	11.2
3.65	1.85	3.19	6.3	8.8	4.11	2.03	3.38	8.5	11.3
3.68	1.90	3.22	6.7	9.2	4.12	2.10	3.32	8.0	11.8
3.68	1.76	3.07	5.7	8.4	4.12	2.06	3.52	9.7	12.5
3.71	1.87	3.06	7.2	8.9	4.15	2.15	3.57	8.5	13.4
3.75	1.87	3.19	6.5	9.7	4.15	2.04	3.43	8.2	11.9
3.76	1.92	3.21	7.1	9.7	4.15	2.06	3.39	8.9	11.5
3.78	1.85	3.18	6.8	8.7	4.15	1.92	3.42	8.8	11.3
3.79	1.94	3.10	6.6	9.5	4.15	2.25	3.46	8.7	12.5
3.80	1.90	3.15	7.1	9.1	4.17	2.04	3.47	8.8	11.8
3.81	1.80	3.21	7.0	8.3	4.17	2.06	3.44	7.8	12.3
3.82	1.97	3.15	6.8	9.6	4.17	2.03	3.47	9.8	11.9
3.85	1.80	3.24	7.7	9.4	4.20	2.17	3.38	8.3	12.7
3.86	1.88	3.19	6.1	9.4	4.21	2.12	3.53	10.2	13.1
3.87	1.90	3.18	6.2	9.6	4.21	2.08	3.44	9.0	14.3
3.88	1.84	3.14	7.4	9.7	4.25	2.06	3.57	9.4	12.5
3.88	1.91	3.25	6.8	9.6	4.25	2.12	3.61	11.0	12.9
3.90	1.89	3.27	6.4	9.9	4.25	2.13	3.57	10.4	12.5
3.91	1.90	3.25	8.2	9.9	4.27	2.27	3.53	10.5	13.1
3.93	1.99	3.20	7.5	10.4	4.31	2.03	3.69	9.9	13.2
3.93	1.97	3.22	7.5	10.5	4.31	2.09	3.39	8.0	12.0
3.94	1.89	3.13	6.6	10.1	4.31	2.31	3.63	11.4	14.4
3.95	1.86	3.39	7.4	10.4	4.31	2.12	3.38	8.0	12.4
3.95	1.91	3.26	7.7	10.3	4.32	2.08	3.61	9.8	13.3
3.96	1.94	3.20	6.6	9.7	4.33	2.17	4.03	9.5	13.2
3.96	1.84	3.23	7.8	9.5	4.38	2.13	3.48	10.4	13.2
3.97	1.93	3.34	6.5	10.3	4.39	2.27	3.54	10.1	14.3
3.98	1.93	3.38	7.8	10.2	4.40	2.24	3.69	9.0	15.4
4.02	1.91	3.33	8.7	10.9	4.41	2.18	3.67	12.7	14.9
4.03	1.98	3.29	7.2	10.7	4.50	2.16	3.72	9.6	14.8

4.50	2.24	3.68	12.0	16.0	4.90	2.30	3.94	12.3	17.9
4.52	2.10	3.71	10.1	14.7	4.90	2.43	4.02	16.0	19.2
4.54	2.23	3.71	11.2	15.1	4.91	2.60	4.17	14.6	21.4
4.54	2.24	3.80	9.8	15.1	4.91	2.49	3.99	12.8	19.6
4.55	2.09	3.65	10.7	14.0	4.92	2.50	4.13	11.7	20.1
4.58	2.37	3.72	10.9	15.8	4.97	2.41	4.03	14.3	19.0
4.58	2.35	3.80	12.1	16.9	4.98	2.55	4.03	13.3	20.9
4.65	2.37	3.77	12.1	16.9	5.07	2.47	4.23	15.8	21.1
4.68	2.32	3.86	12.1	16.5	5.10	2.38	4.05	12.9	20.1
4.78	2.41	3.88	11.5	18.1	5.13	2.40	4.14	12.3	20.5
4.80	2.47	3.96	13.1	19.5	5.17	2.44	4.11	14.2	20.4
4.80	2.23	3.86	11.7	16.4	5.30	2.67	4.24	20.3	23.8
4.83	2.44	4.03	13.7	18.4	5.50	2.60	4.43	17.6	24.8
4.90	2.40	3.90	11.9	18.3					

TABLE 5. Turesima-Mati, Okayama-Ken.
Collected on Nov. 19, 1932.

Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Volume in cc.	Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Volume in cc.
2.76	1.37	2.21	2.8	3.6	3.12	1.58	2.60	4.1	5.2
2.80	1.31	2.29	2.8	3.7	3.13	1.49	2.54	3.6	5.2
2.81	1.36	2.37	3.3	4.0	3.14	1.57	2.62	4.2	5.3
2.81	1.36	2.33	2.8	3.8	3.15	1.54	2.57	4.0	5.2
2.85	1.46	2.36	3.5	4.2	3.15	1.48	2.54	3.7	4.9
2.87	1.43	2.35	3.3	4.0	3.17	1.59	2.63	3.9	4.6
2.89	1.35	2.36	3.2	3.9	3.17	1.53	2.58	4.0	5.2
2.89	1.44	2.52	3.0	4.4	3.18	1.60	2.59	4.0	5.3
2.91	1.47	2.41	3.7	4.4	3.19	1.62	2.63	4.4	5.6
2.92	1.45	2.42	3.3	4.3	3.19	1.55	2.57	4.0	5.4
2.92	1.37	2.34	3.1	3.7	3.19	1.72	2.64	4.9	5.7
2.92	1.41	2.39	3.4	4.3	3.20	1.52	2.65	4.1	5.3
2.92	1.41	2.40	3.0	4.2	3.20	1.59	2.62	4.1	5.4
2.95	1.36	2.42	3.3	4.2	3.20	1.62	2.60	4.0	5.5
2.97	1.49	2.43	3.6	4.5	3.22	1.75	2.63	4.4	6.2
2.99	1.40	2.42	3.5	4.3	3.22	1.66	2.67	5.2	5.0
3.00	1.49	2.40	3.5	4.6	3.23	1.61	2.61	4.1	5.4
3.00	1.49	2.50	3.6	4.6	3.24	1.60	2.72	4.5	6.0
3.01	1.43	2.43	3.3	4.5	3.24	1.62	2.56	4.1	5.6
3.01	1.44	2.40	3.6	4.4	3.25	1.56	2.60	4.1	5.4
3.01	1.44	2.44	3.6	4.4	3.25	1.63	2.70	4.2	6.0
3.02	1.50	2.50	3.3	4.7	3.25	1.62	2.70	4.7	6.4
3.02	1.45	2.48	3.7	4.7	3.28	1.67	2.66	4.5	5.7
3.03	1.55	2.53	3.9	5.0	3.29	1.70	2.68	4.3	6.1
3.04	1.53	2.55	4.0	4.8	3.30	1.68	2.68	4.2	6.0
3.05	1.45	2.52	3.5	4.5	3.30	1.66	2.67	4.1	5.9
3.05	1.53	2.47	3.6	5.0	3.30	1.65	2.70	4.3	6.1
3.06	1.52	2.58	3.8	5.1	3.30	1.60	2.73	4.1	6.0
3.07	1.51	2.49	4.0	4.9	3.32	1.62	2.81	4.8	6.1
3.07	1.50	2.56	3.6	4.8	3.32	1.72	2.72	5.7	6.3
3.07	1.52	2.51	3.7	4.8	3.34	1.69	2.70	4.3	6.2
3.08	1.53	2.47	3.9	5.2	3.34	1.70	2.71	4.7	6.2
3.08	1.53	2.52	3.7	5.0	3.34	1.59	2.69	4.2	5.8
3.10	1.56	2.60	4.1	5.0	3.34	1.69	2.74	4.6	6.6
3.10	1.57	2.58	4.0	5.3	3.35	1.69	2.79	4.8	6.3
3.10	1.68	2.60	4.3	5.6	3.36	1.62	2.72	4.5	6.0
3.11	1.46	2.59	3.6	4.9	3.37	1.76	2.84	5.5	6.6
3.11	1.53	2.55	3.8	5.1	3.38	1.58	2.78	4.7	6.2

3.38	1.71	2.82	5.4	6.7	3.64	1.78	3.06	5.9	8.0
3.38	1.71	2.72	4.9	6.4	3.66	1.90	3.02	5.8	8.5
3.40	1.73	2.74	4.6	6.7	3.68	1.87	2.99	6.6	8.6
3.40	1.72	2.78	4.7	6.7	3.68	1.81	3.00	5.9	7.9
3.41	1.74	2.77	5.4	6.7	3.69	1.88	2.96	5.5	8.2
3.42	1.71	2.85	4.9	6.8	3.71	1.85	3.04	6.4	8.7
3.42	1.70	2.87	5.0	6.9	3.71	1.92	3.08	6.1	8.9
3.43	1.74	2.85	4.8	6.9	3.72	1.88	3.06	5.6	8.6
3.44	1.79	2.75	4.5	6.8	3.73	1.84	3.08	6.0	8.7
3.45	1.74	2.91	6.0	7.2	3.73	1.78	3.02	5.6	8.1
3.46	1.78	2.81	4.6	7.0	3.74	1.95	3.03	6.3	9.1
3.46	1.75	2.91	5.2	7.0	3.77	2.06	3.18	5.9	9.9
3.47	1.69	2.83	4.4	6.8	3.78	1.96	3.06	6.7	9.0
3.48	1.69	2.85	4.8	7.0	3.79	1.96	3.11	6.1	9.3
3.48	1.81	2.92	5.5	7.5	3.80	1.84	3.10	6.1	8.7
3.48	1.74	2.77	5.2	6.9	3.81	1.81	2.97	5.8	8.3
3.49	1.70	2.89	5.8	7.1	3.81	1.84	3.14	5.0	8.8
3.50	1.70	2.85	5.3	7.1	3.83	1.90	3.11	7.2	9.1
3.50	1.69	2.75	4.9	6.8	3.84	2.11	3.15	7.4	10.5
3.51	1.72	2.97	5.1	7.3	3.84	1.91	3.16	6.4	9.4
3.51	1.72	2.86	5.8	7.0	3.85	2.03	3.20	7.5	10.1
3.51	1.75	2.98	5.7	7.4	3.86	1.96	3.20	7.0	9.2
3.52	1.84	2.87	5.8	7.4	3.87	1.90	3.10	6.2	9.3
3.53	1.76	2.91	5.3	7.2	3.89	1.94	3.18	7.0	9.9
3.53	1.84	2.90	5.3	7.9	3.90	1.98	3.31	7.9	10.4
3.53	1.68	2.80	5.2	6.9	3.91	1.91	3.19	7.5	9.8
3.55	1.75	2.89	5.6	7.4	3.92	2.03	3.23	6.9	10.0
3.55	1.70	2.91	5.0	7.2	3.93	2.03	3.20	6.7	10.3
3.56	1.86	2.95	5.8	7.7	3.95	2.05	3.23	6.4	10.5
3.58	1.69	2.86	5.4	7.1	3.97	1.99	3.22	7.1	10.3
3.58	1.80	2.88	5.2	7.7	3.98	2.03	3.23	8.0	10.6
3.61	1.84	2.97	6.4	8.0	4.01	1.98	3.10	6.0	9.8
3.63	1.86	3.02	5.5	8.5	4.13	2.14	3.37	7.1	12.5
3.63	1.89	3.05	5.5	8.8	4.18	2.17	3.39	7.9	12.2

TABLE 6. Takamatu-Mati, Isikawa-Ken.

Collected on Oct. 14, 1932.

Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Volume in cc.	Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Volume in cc.
3.71	1.65	3.03	7.6	7.6	4.60	1.93	3.54	12.7	12.6
4.05	1.86	3.25	9.8	9.8	4.61	1.90	3.60	12.1	12.7
4.30	1.96	3.44	11.1	11.8	4.61	2.05	3.62	13.5	13.6
4.35	1.95	3.41	11.8	12.0	4.62	2.08	3.63	13.2	13.9
4.35	1.99	3.35	11.0	11.5	4.64	2.03	3.66	13.2	13.9
4.41	2.11	3.61	13.7	13.7	4.65	2.17	3.75	15.2	15.6
4.43	1.97	3.43	12.0	12.0	4.65	2.06	3.68	13.9	14.2
4.47	1.96	3.45	11.8	12.2	4.66	2.01	3.60	12.9	13.3
4.49	1.94	3.45	12.4	12.4	4.69	2.04	3.73	14.3	14.2
4.51	2.04	3.65	12.7	13.2	4.71	2.12	3.62	14.0	14.4
4.51	2.08	3.56	13.5	13.7	4.71	2.08	3.72	14.3	14.9
4.52	1.98	3.44	11.6	12.0	4.72	2.18	3.67	15.1	15.2
4.52	2.00	3.48	12.0	12.6	4.73	2.08	3.70	14.4	14.7
4.52	2.03	3.53	12.7	12.8	4.73	2.10	3.62	14.3	14.5
4.53	2.00	3.53	13.4	12.9	4.76	2.16	3.78	15.3	15.3
4.54	2.01	3.62	13.2	13.5	4.77	2.31	3.86	16.2	16.3
4.59	2.00	3.53	13.0	13.0	4.78	2.12	3.67	14.6	14.7
4.60	2.04	3.62	13.5	13.5	4.79	2.18	3.71	14.4	15.1

Length in cm.	Depth in cm	Height in cm	Weight in gm	Volume in cc.	Length in cm.	Depth in cm.	Height in cm	Weight in gm.	Volume in cc
4.79	2.15	3.63	15.2	15.0	5.02	2.38	3.94	17.8	19.0
4.79	2.29	3.70	15.7	16.1	5.03	2.25	3.80	15.5	17.1
4.81	2.08	3.72	15.1	16.0	5.03	2.28	4.06	17.7	18.6
4.81	2.28	3.83	16.6	16.8	5.06	2.13	3.81	16.0	15.8
4.85	2.29	3.76	16.8	16.6	5.06	2.21	3.89	17.0	17.9
4.85	2.17	3.81	15.3	16.0	5.08	2.36	3.94	18.2	19.3
4.85	2.34	3.77	17.4	17.5	5.09	2.42	3.92	18.7	19.1
4.85	2.16	3.76	15.6	15.9	5.09	2.24	3.92	17.9	17.6
4.85	2.15	3.82	15.8	15.5	5.10	2.25	3.86	18.2	17.6
4.87	2.11	3.62	14.3	15.0	5.10	2.29	4.00	17.2	18.7
4.88	2.32	3.77	16.6	17.7	5.10	2.29	3.96	18.0	18.8
4.88	2.27	3.93	18.2	17.7	5.11	2.30	3.83	17.1	17.7
4.90	2.14	3.83	15.7	15.6	5.12	2.35	4.01	18.5	18.9
4.90	2.27	3.72	16.1	16.5	5.12	2.33	3.91	18.3	18.6
4.90	2.32	3.86	18.1	17.7	5.13	2.31	3.99	18.7	19.3
4.92	2.33	3.84	18.3	17.4	5.15	2.38	4.05	18.9	19.3
4.94	2.20	3.91	15.6	16.4	5.16	2.30	4.13	20.0	19.3
4.95	2.20	3.80	16.7	16.7	5.17	2.50	4.07	21.1	20.9
4.96	2.30	3.85	16.7	17.1	5.18	2.41	4.14	21.1	21.1
4.97	2.21	3.86	17.3	17.3	5.21	2.26	3.90	18.2	18.6
4.97	2.29	3.80	16.6	17.4	5.22	2.45	3.94	19.4	20.1
4.98	2.19	3.88	15.4	17.0	5.25	2.30	4.01	19.2	19.6
4.98	2.20	3.78	16.1	16.4	5.26	2.30	3.93	17.9	18.9
4.99	2.20	3.88	16.5	16.3	5.30	2.39	3.95	18.9	20.0
4.99	2.20	3.73	16.2	16.4	5.31	2.40	4.16	20.8	21.6
5.00	2.21	3.95	18.0	17.7	5.33	2.46	4.08	20.2	21.5
5.00	2.31	3.91	18.5	18.1	5.33	2.29	4.00	18.6	19.4
5.01	2.29	3.86	17.0	17.5	5.34	2.40	4.09	21.3	21.1
5.01	2.27	3.90	16.7	17.5	5.39	2.40	4.03	19.5	20.9
5.01	2.24	3.89	16.9	17.5	5.41	2.56	4.26	23.0	23.2
5.01	2.26	3.94	18.4	17.9	5.43	2.41	4.11	20.5	21.4
5.02	2.26	3.97	19.0	17.1	5.45	2.41	4.19	21.4	22.3
5.02	2.16	3.90	17.1	17.1	5.48	2.35	4.16	20.8	21.4
5.02	2.32	3.92	17.0	18.7	5.49	2.35	4.08	21.3	21.4

GANGLION CELLS IN THE HEART OF *LIGIA EXOTICA* (ROUX)

By

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Mitsui Institute of Marine Biology, Shimoda, Japan

(With four figures)

(Received July 17, 1934)

The innervation of the heart of *Crustacea* have been already studied by ALEXANDROWICZ ('26, '32) and NEWMYWAKA ('28). But their studies were on the heart of Decapods. There is no paper, as far as I am aware, on the studies of the innervation of the Isopods-heart.

Ligia exotica (ROUX) belongs to *Oniscoidae* of *Isopoda*, *Crustacea*, and is commonly found on the sea-shore of Japan.

The present paper deals with the innervation of the heart of *Ligia exotica* which has been observed since 1933 at the Mitsui Institute of Marine Biology.

MATERIAL AND METHOD

The material, *Ligia exotica* (ROUX), was collected from the beach in the neighbourhood of the Institute.

The thorax and abdominal segments were cut away carefully so as not to injure the heart and the arteries. Then the heart with surrounding tissues was dissected out and fixed in BOUIN's-, FLEMMING's-, ZENKER's-solution, acetic sublimate, or pyridine. The materials were embedded in paraffin and sectioned serially in the thickness of 8~12 μ . For the staining, DELAFIELD's haematoxylin with eosin, HEIDENHAIN's iron-haematoxylin, MALLORY's triple connective staining mixture, and phosphotungstic haematoxylin were applied. Vital staining with methylene blue and Rongalit white were also tried, without success for the muscles of the heart contain too many granules.

OBSERVATION

The nerve of this Isopode runs along the ventro-median line of the body. The sixth and seventh thoracic ganglion contact each other. The abdominal ganglion is the largest except the brain and gives off six pairs

of nerve branches (Fig. 1). Anterior four pairs of nerve branches run side-way but other two pairs run to posterior part of the body along the digestive canal. Multipolar nerve cells are situated on the ventral surface of

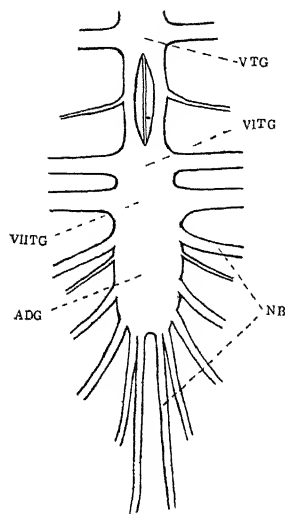


Fig 1 Diagram to show the abdominal ganglion. ADG. Abdominal ganglion; NB. nerve branches, TG. thorax ganglion.

the ganglion and measure about $33\sim40\mu$ in width, $58\sim75\mu$ in length. The nerve cell contains a large nucleus which has one or two clear nucleolus. The nucleus of the nerve cell measures about 18μ in diameter.

Along the dorso-median line of the body, a thin-walled tube runs straightly. The posterior part of this tube swells and ends blindly. This swelled part of the tube is the heart of the worm. The heart ends in the fifth abdominal segment blindly and gives off side-way four pairs of arteries. From the most anterior part of the heart, a stout artery, median artery, starts towards the anterior of the thorax and it reaches to the head region. On the dorsal wall of the heart are two ostia.

The walls of the side-arteries and anterior portion of the median artery are very thin and consist of one-layered tissue. The wall of

the posterior part of the median artery is thicker than that of other arteries but very thinly muscular in comparison with that of the heart. At the branching place of each artery from the heart, a muscular clap was seen. The heart muscles are striated and almost all of them run rectangular to the long axis of the heart.

A nerve fibre bundle runs straightly along the median line of the heart and surpasses almost all of the length of the heart. The nerve fibre bundle is situated in the dorsal wall of the heart to its inner surface. This nerve bundle may be called "ganglionic trunk" as named by ALEXANDROWICZ in Decapods.

The ganglion cells in the heart were discovered (Fig 2), and their number was found to be constant, six ganglion cells. These ganglion cells arrange themselves straightly along the nerve fibre bundle in the heart. The third and fourth ganglion cells from the anterior are connected with each other (Fig. 2). Figure 3 diagrammatically illustrates the distribution of these ganglion cells in the heart.



Fig 2 Photograph to show the direct contact of the third and fourth ganglion cells in the heart Lo gitudinal section $\times 320$

The ganglion cell is multipolar in shape, takes a dark violet or black colour with haematoxylin staining, and measures about 35μ in width and $63\sim 70\mu$ in length. Nucleus of the ganglion cell is very large, round in shape and measures about 17μ in diameter. One clear nucleolus in the center of the nucleus was found in each case. I could not decided how the nerve processes of the ganglion cell end in the muscles of the heart.

The nerve fibres which enter the heart seems to be of two pairs. The first pair of nerve fibre originates from the first nerve branch of the abdominal ganglion and the second fibre from the third branch of the same ganglion. They pass between the muscle bundles and enter the heart at the mid-dorsal wall of the heart (Fig 4). The first pair of nerve fibre communicates with that of the heart at the anterior portion of

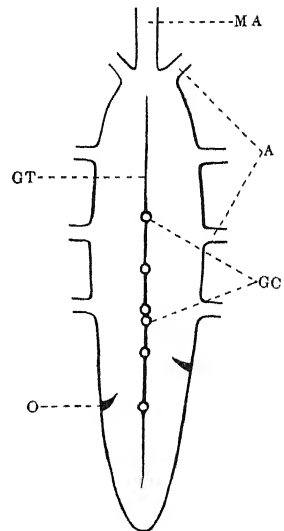


Fig 3 Diagram to show the distribution of the ganglion cells in the heart A arteries; GC ganglion cells; GT. ganglionic trunk, MA median artery, O ostium

the first ganglion cell of the heart and the second pair at the middle portion between the second and third ganglion cells. Near the heart, the first nerve fibre possesses two nerve cells but the second fibre possesses

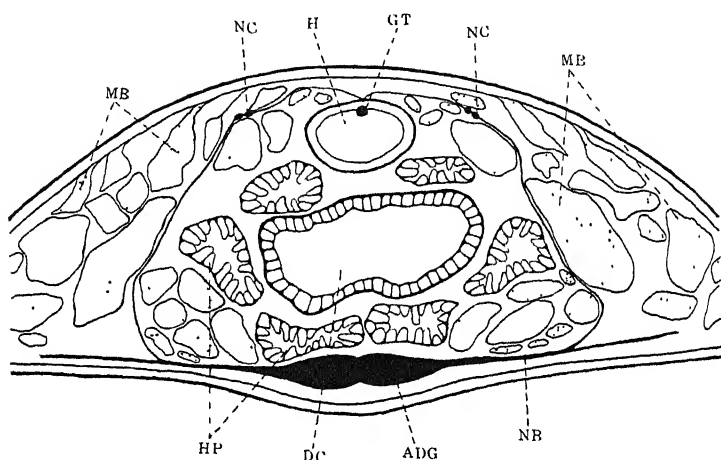


Fig 4. Semidiagrammatical figure to show the nerve fibre which enter the heart. ADG abdominal ganglion, DC digestive canal; GT ganglionic trunk, H heart, HP hepatopancreas, MB muscle bundles, NB nerve branch; NC nerve cells.

only one cell on each nerve fibre. These nerve cells situated along the nerve fibre and are elongated, bipolar in shape and a little smaller than that of the abdominal ganglion.

SUMMARY

1. The gross anatomy of the abdominal ganglion and the heart is given.
2. A nerve fibre bundle which contains six ganglion cells runs along the dorso-median line of the heart.
3. Ganglion cells in the heart are multipolar in shape and arrange themselves straightly along the nerve fibre bundle in the heart.
4. Two pairs of nerve fibre enter the heart from the mid-dorsal wall of the heart. Near the heart, the first pair of nerve possesses two nerve cells but the second only one cell on each fibre.

LITERATURE

- ALEXANDROWICZ, J. S. 1926. The Innervation of the Heart of Cockroach (*Periplaneta orientalis*). Journ. Comp. Neur., Vol. XLI.
- 1932 The Innervation of the Heart of the Crustacea. I. Decapoda. Quart. Journ. Micr. Science, Vol. LI, No. 1.
- NEWMYWAKA, G. A. 1928. Zur Frage über die Innervation der Herzens beim Flusskrebs (*Potamobius astacus* L.). Zool. Anz., Vol. LXXIX.
- GOTO, S and TERAOKA, A. 1911 Anatomy of *Ligia exotica* (Roux). Dobutsugaku-zasshi, Vol. XXIII.

REPORT ON THE FRESH-WATER SPONGES OBTAINED FROM HOKKAIDÔ

By

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(With Pls. IV-VII and 15 text-figures)

(Received July 25, 1934)

The fauna of the fresh-water sponges of Hokkaidô seem have hitherto remained almost unknown.

In the Autumn of 1933, I made a trip to Hokkaidô and tried to collect the sponges of that group in several lakes, ponds and rivers.

Thus I was able to obtain eight species in all which are shown in the following list. Of these eight species, six are identical with those previously known, while the remaining two are here described for the first time.

In the present report I shall deal with these species.

1. *Spongilla lacustris* (LINNÉ).
2. *Spongilla shikaribensis*, n. sp.
3. *Spongilla fragilis* LEIDY.
4. *Spongilla akanensis*, n. sp.
5. *Ephydatia fluviatilis* (LINNÉ).
6. *Ephydatia mülleri* (LIEBERKÜHN).
7. *Ephydatia mülleri* var. *japonica* (HILGENDORF).
8. *Heteromeyenia baileyi* var. *petri* (LAUTERBORN).

It is my pleasant duty to express my hearty thanks to Professor HÔZAWA who has courteously helped me many times during the course of my investigation.

1. *Spongilla lacustris* (LINNÉ)

(Pl. IV, Figs. 1, 2, 3, 4, 5, 6, 7, 8, Pl. VII, Fig. 29; Text-figs. 1, 2).

Spongia lacustris, LINNÉ 1759, p. 1348.

Spongia canalium, SCHRÖTER 1788, pp. 149-158.

Spongia fibrillosa, ESPER 1794, p. 235.

Spongilla ramosa, LAMARCK 1816, p. 100.

Tupha lacustris, THIENEMANN 1828, p. 16.

Spongilla erinaceus, EHRENBERG 1841, p. 363.

Spongilla dawsoni, BOWERBANK 1863, pp. 467-468.

Spongilla lacustrioides, POTTS 1879.

Spongilla abortiva, POTTS 1880.

Spongilla mutica, POTTS 1880.

Spongilla montana, POTTS 1880, p. 357.

Spongilla multiforis, CARTER 1881, pp. 88-89.

Spongilla lacustris, CARTER 1881, p. 87; POTTS 1887, p. 186;

ANNANDALE and KAWAMURA 1916, pp. 3-5.

This sponge grows in stagnant or running water, being attached to the logs, twigs, stones and some other objects placed in water at a depth of about 1-5 meters.

In form (Pl. IV, Figs. 1-8), it may sometimes be massive without any projecting branches (Fig. 4), but in general, it is made up of a relatively thin encrusting layer, provided with a number of vertically projecting branches.

These branches are variable in length and thickness and are cylindrical in form, being broad in the basal part and tapering towards the tip. In some cases they may be flattened instead of being cylindrical and may form a complicated network by anastomosing (Fig. 2).

The outer surface of the sponge is rather bristly on account of the radiating spicules.

The living sponge is usually soft and rather fragile and is covered with a well defined external dermal membrane.

The sponge becomes very brittle when it is dried.

The sponge grown in such a place as to receive sufficient sun-light looks bright green, whilst that grown in a dark place or in such a place as to receive only faint sun-light is yellowish, greyish or whitish instead of being green.

Each of the oscula, in general, is rather inconspicuous, but sometimes it may grow larger, surrounded by a well-defined border. Usually it is protected by a transparent collar of conical form.

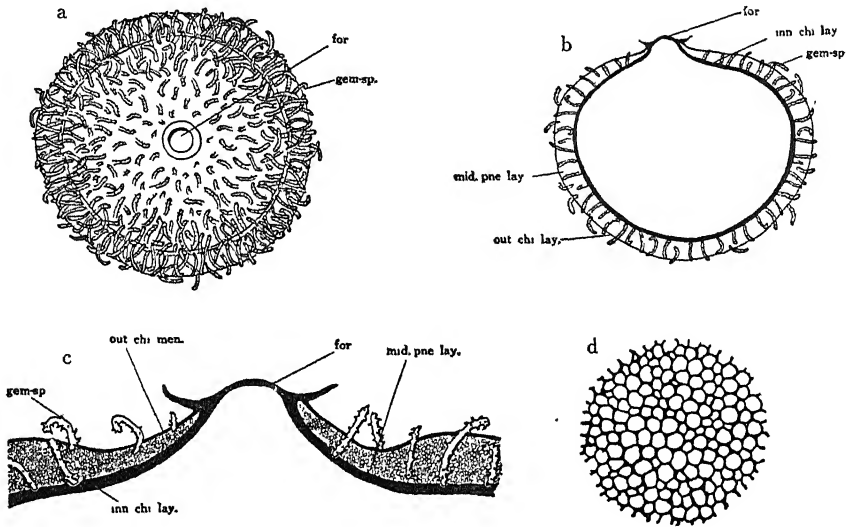
Though the dermal pores (=ostia) are great in number, they are not large and conspicuous.

There are two groups of fibres which form the skeleton of the sponge.

The one group consists of radiating or vertical fibres. They are relatively slender but are well-defined. Each of these fibres is composed of 5-20 spicules in cross-section cemented together by a horny substance or spongin. The other group is transverse fibres. They are more or less inconspicuous than the first, each of the fibres being composed of 1-3

spicules in cross-section.

Gemmules (Text-fig. 1). The gemmules are formed abundantly and are found freely in the interstices of the skeleton. They occur not only in the main body but also in the branches projecting from it.



Text-fig. 1. *Spongilla lacustris* (L.)

a, Gemmule, showing a foramen in the center. b, Section of a gemmule through the foramen. c, Sagittal-section of the foramen. d, A part of the pneumatic layer (a, b $\times 60$, c $\times 180$; d $\times 600$). *for.*, foramen (=foraminal aperture); *gem-sp.*, gemmule-spicule; *inn. chi. lay.*, inner chitinous layer; *mid. pne. lay.*, middle pneumatic layer; *out chi. lay.*, outer chitinous layer.

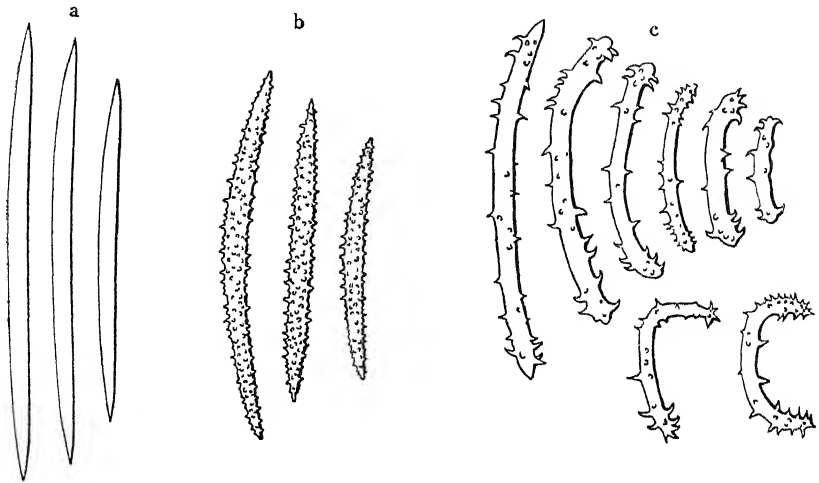
They are spherical in form (Text-fig. 1, a) and are of considerable size, their greatest diameter attaining $550\text{--}700\ \mu$ (average $617\ \mu$).

In colour they are yellowish or brownish.

Each gemmule is covered by a thick pneumatic coat (Text-fig. 1, b, c) and is provided with a single foramen or foraminal aperture surrounded by a cup-like structure of $85\text{--}107\ \mu$ diameter (Pl. VII, Fig. 29). The diameter of the foramen is $45\text{--}50\ \mu$. In the pneumatic coat the gemmule-spicules are placed vertically or parallel to the surface of the coat.

As a rule the pneumatic coat of the gemmule is composed of three layers; viz. 1) the inner chitinous layer, $6\text{--}7\ \mu$ thick; 2) the middle pneumatic layer which is composed of numerous small air-chambers (Text-fig. 1, d) and is comparatively thick, being $30\text{--}40\ \mu$ thick; and 3) the outer thin chitinous membrane containing the gemmule-spicules, is $1\text{--}4\ \mu$ thick.

Spicules (Text-fig. 2). The skeleton-spicules (Text-fig. 2, a) are relatively long but slender. They are smooth, straight or slightly curved, and are gradually and sharply pointed at the extremities.



Text-fig. 2. *Spongilla lacustris* (L.).

a, Skeleton-spicules. b, Flesh spicules. c, Gemmule-spicules (a $\times 180$, b, c $\times 600$).

They are $270\text{--}350\ \mu$ (average $308\ \mu$)¹⁾ long and $10\text{--}14\ \mu$ (average $12.2\ \mu$) thick in the thickest portion.

The free microscleres or flesh-spicules (Text-fig. 2, b) are found abundantly both in the dermal membrane and in the interstices among the main skeleton. They are short, straight or slightly curved, gradually and sharply pointed at both ends and are densely covered with minute spines. They are $55\text{--}80\ \mu$ (average $66.8\ \mu$) long and $4\text{--}5\ \mu$ (average $4.5\ \mu$) thick at the thickest portion.

The gemmule-spicules (Text-fig. 2, c) resemble the microscleres above mentioned, but on the whole, are stouter and more strongly curved in a hook-like manner. Their ends are rounded or abruptly pointed and are covered with much less number of spines than in the case of flesh-spicules. They are rather variable in size being measured $26\text{--}80\ \mu$ (average $45.4\ \mu$) in length and $2.5\text{--}4.5\ \mu$ (average $3.6\ \mu$) thick at the thickest portion.

Remarks. — This species was first described by LINNÉ in the name of *Spongia lacustris* in the 10th edition of *Systema Naturae*. It seems

¹⁾This average is determined by measuring the mean of 100 examples of the spicules.

to be almost cosmopolitan, being distributed nearly all over the world, and thus many varieties of this sponge were described from various localities. But it has not been hitherto reported from Central and South America.

From Japan ANNANDALE and KAWAMURA reported in detail on this sponge.

Among the lakes in Hokkaidô where I have collected this kind of sponge, Lake Akan is most remarkable, as there the sponge grows very thick and high thus giving a splendid luxuriant view. Some of these sponges attain the height of more than half a metre (Pl. I, Fig. 1).

Previously known distribution.—Widely distributed in Asia, Australia, Europe, British Isles, Africa, North America. Japan: Lake Noziri, Lake Kizaki and Lake Nakatsuna, Province of Shinano, Lake Biwa, a small lake at Komatsu, Lake Yogo, Province Ômi (ANNANDALE and KAWAMURA).

Localities.—Shimekirinuma, Province of Rikuzen (collected by Mr. Z. KADOTA); The River Hirose, Province of Rikuzen; A canal of Ôyô Park, Province of Mutsu; Hakamagata Pond near Goshogawara, Province of Mutsu; Tsutanuma near Mt. Hakkôda, Province of Mutsu (collected by Mr. Y. HORIKAWA); Lake Akan, Province of Kushiro, Hokkaidô; Lake Abashiri, Province of Kitami, Hokkaidô; Utonaitonuma near Tomakomae, Province of Iburî, Hokkaidô (collected by Dr. T. UCHIDA); Konuma near Ônumakôen, Province of Oshima, Hokkaidô.

2. *Spongilla shikaribensis*, n. sp.

(Pl. V, Figs. 9, 10, 11; Pl. VII, Fig. 30, Text-figs. 3, 4)

This sponge grows on the surface of submerged logs, roots, branches, twigs, pebbles, stones, rocks, pillars of bridges, and other objects both in quiet and rapidly flowing water, at a depth of 0.5–5 metres.

The external form of this sponge when grown in stagnant water is that of a relatively thin encrusting layer, from which a number of vertical, elongate, cylindrical, long or short projections or tubercles usually arise (Pl. V, Figs. 9, 10); but it may be a thin, flat, filmy patch attaching to the surface of other objects when it is growing in rapidly flowing water (Pl. V, Fig. 11).

This sponge is, as a rule, soft and very fragile in consistency.

The colour of the sponge in life is bright green, when grown in a good light of the sun; but when it is grown in a faint light or in the shade, the green colour often disappears and the sponge turns yellowish or whitish.

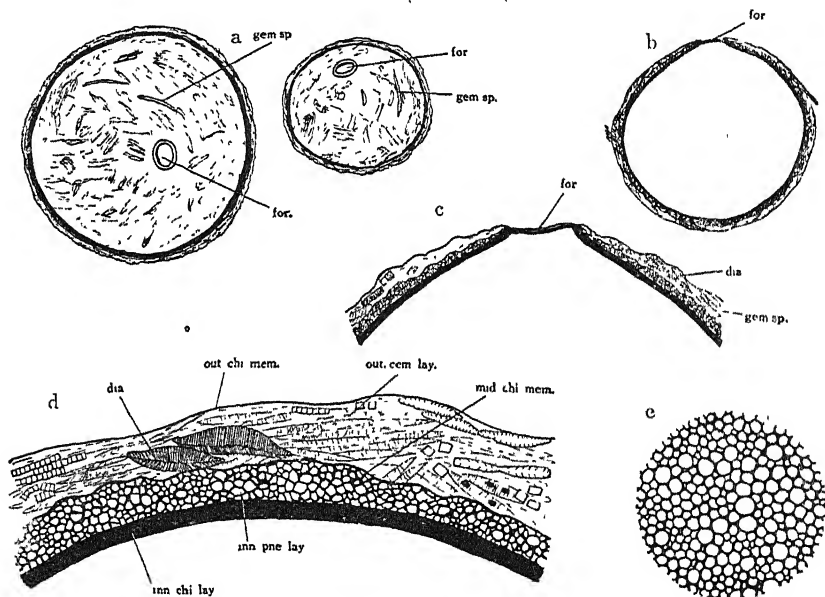
The oscula and pores are present abundantly but they are never large or conspicuous.

The skeleton fibres which are mainly composed of vertical fibres, are rather poorly defined and form a very loose irregular network in the gemmule region.

The vertical fibres which are composed of 2-5 or more skeleton-spicules in cross-section, run longitudinally in the central part of the sponge, and they may change their direction vertically against the surface of the sponge. The distal ends of the vertical fibres usually project externally beyond the dermal membrane.

The transverse fibres which are dispersed usually among the vertical fibres, are very poorly developed, each fibre being composed of 1-2 spicules in cross-section.

Gemmules (Text-fig. 3). The gemmules are freely produced in the



Text-fig 3 *Spongilla shikaribensis*, n. sp.

a, Gemmules. b, Section of a gemmule through the foramen. c, Sagittal-section of the foramen. d, Vertical-section of the gemmule-coat. e, A part of the pneumatic layer. (a, b $\times 60$; c $\times 180$; d, e $\times 600$).

dia., diatom; for., foramen; gem-sp., gemmule-spicule; inn. chi. lay., innermost chitinous layer; inn pne lay., inner pneumatic layer; mid chi. mem., middle chitinous membrane; out. cem. lay., outer cementing layer; out. chi. mem., outermost chitinous membrane

interstices of the skeleton, and are especially abundant at the base of the sponge.

They are spherical in form, usually brown or yellow in colour, and are very variable in size being $200\text{--}700\ \mu$ (average $490\ \mu$) in diameter (Text-fig. 3, a).

Each gemmule is usually covered with a very characteristic coat which is composed of the following three layers and two membranes (Text-fig. 3, d); viz. 1) the innermost chitinous layer which is commonly found in other species too, is in this case homogenous and brown in colour, being $3\text{--}5\ \mu$ thick; 2) the inner pneumatic layer which is composed of granular "air-cells" (Text-fig. 3, e), is rather transparent, usually undulated and very variable in thickness, being $5\text{--}12\ \mu$ thick; 3) the middle chitinous membrane is very thin and is undulating, existing between the inner pneumatic layer and the following outer cementing layer, and is not more than $1\ \mu$ thick; 4) the outer cementing layer is very remarkable in appearance, composed of hyaline ground substance, and is $10\text{--}20\ \mu$ thick. In this layer some minute foreign objects such as shells or crusts of various diatoms, plankton and the gemmule-spicules are imbedded without any definite orientation; sometimes this layer may be a common covering of the two or more gemmules; and 5) the outermost chitinous membrane which is very thin, and not more than $0.5\ \mu$ thick. Each gemmule has a single foramen which is not protected by any tubule or cup-like structure, except for a thin chitinous operculum placed at the orifice (Pl. VII, Fig. 30). The diameter of the foramen is $40\text{--}55\ \mu$.

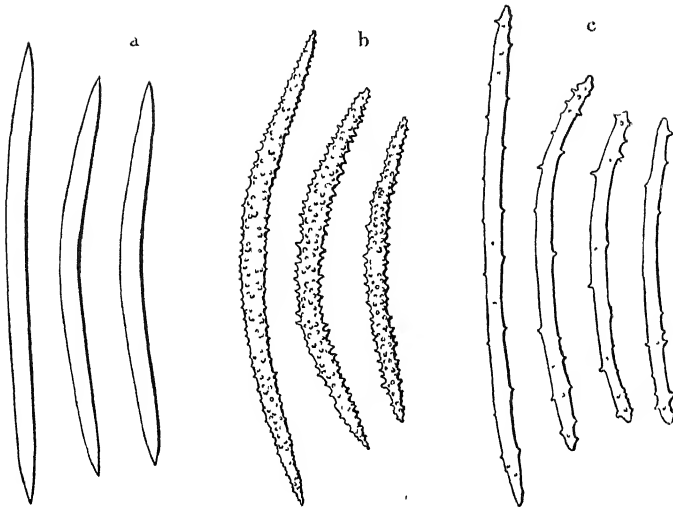
Spicules (Text-fig. 4). The skeleton-spicules (Text-fig. 4, a) are rather slender, very smooth, straight or slightly curved, gradually and sharply pointed at the extremities.

They are relatively few in number and are of moderate size, being $240\text{--}350\ \mu$ (average $293\ \mu$) long and $13\text{--}15\ \mu$ (average $13.9\ \mu$) thick in the thickest portion

The flesh-spicules (Text-fig. 4, b) are small, slightly or strongly curved, gradually tapering towards both ends, densely covered with minute spines.

They are variable in size and are very numerous both in the dermal membrane and in the sponge tissue, being $61\text{--}100\ \mu$ (average $80.1\ \mu$) long and $3\text{--}5\ \mu$ (average $4.1\ \mu$) thick in the thickest portion.

The gemmule-spicules (Text-fig. 4, c) are slender, cylindrical, straight or curved, bluntly pointed or rounded at both ends, covered with a few dispersed minute spines. They are few in number and are very variable in size, being $50\text{--}101\ \mu$ (average $75.7\ \mu$) long and $3\text{--}4\ \mu$ (average $3.4\ \mu$)



Text-fig. 4. *Spongilla shikaribensis*, n. sp.

a, Skeleton-spicules. b, Flesh-spicules. c, Gemmule-spicules (a $\times 180$, b, c $\times 600$)

thick in the thickest portion.

Remarks.— This new species seems to be closely allied to *Spongilla lacustris* (L.) resembling especially in form the skeleton-spicules, flesh-spicules, and gemmule-spicules. But the following characteristics of *S. shikaribensis* will easily distinguish this species from *S. lacustris*.

First, *S. shikaribensis* has poorly-developed skeleton fibres, and relatively few gemmule-spicules, but it has extraordinarily numerous flesh-spicules distributed through the whole of the sponge body. Secondly, the foramen of the gemmule is not protected by any cup-like or tubular structures which are commonly found in other species of the same genus. Thirdly, the coat of the gemmule is very remarkable in structure being provided with a special cementing layer covering the outer surface of the ordinary pneumatic layer.

Locality.— Lake Shikaribetsu, Province of Tokachi, Hokkaidô.

3. *Spongilla fragilis* LEIDY

(Pl. V, Figs 12, 13, 14, 15; Pl VII, Fig 31; Text-figs. 5, 6).

Spongilla fragilis, LEIDY 1851, p. 278; POTTS 1887, p. 197; ANNANDALE 1909, pp. 106–107; ANNANDALE and KAWAMURA 1916, p. 16.

Spongilla lordii, BOWERBANK 1863, p. 466.

Spongilla contecta, NOLL 1870, p. 173.

Spongilla Lieberkühnii, BROTHERRUS 1876, p. 13.

Spongilla sibirica, DYBOWSKI 1878, p. 53.

Spongilla morgiana, POTTS 1880, p. 330.

Spongilla segregata, POTTS 1880, 1887, p. 202.

Spongilla glomerata, NOLL 1886, p. 682.

This sponge is found both in the quiet and streaming water, such as lake, pond, or river, attached to the surface of submerged logs, pillars of bridges, stones, rocks, branches, twigs, and various objects, at a depth of a few metres.

The external form of this sponge is usually flat (Pl. V, Figs. 12, 14, 15), forming spreading layers or crusts without any projecting branches or conspicuous protuberances. But sometimes it is cylindrical (Pl. V, Fig. 13), spindle-shaped or globular when the sponge grows on twigs, branches, stems of water-weeds or on sandy, gravelly bottoms. In consistence it is moderately hard but fragile.

Its external surface is, as a rule, rugged and covered with a number of small ridges or projections.

It has numerous minute, inconspicuous pores and many small oscula on the surface. From each of these oscula arise a number of radiating canals thus representing a star-shaped figure. These canals are the main exhalant canals gathering into the osculum after running for some distance parallel to the surface of the sponge. In the case of the well-developed specimen (Pl. V, Fig. 13), the oscula are rather large and conspicuous.

The colour of the sponge is usually green when it is grown in a good light, but sometimes it is grey, yellow, brown, or dark brown when it is placed in constant shade.

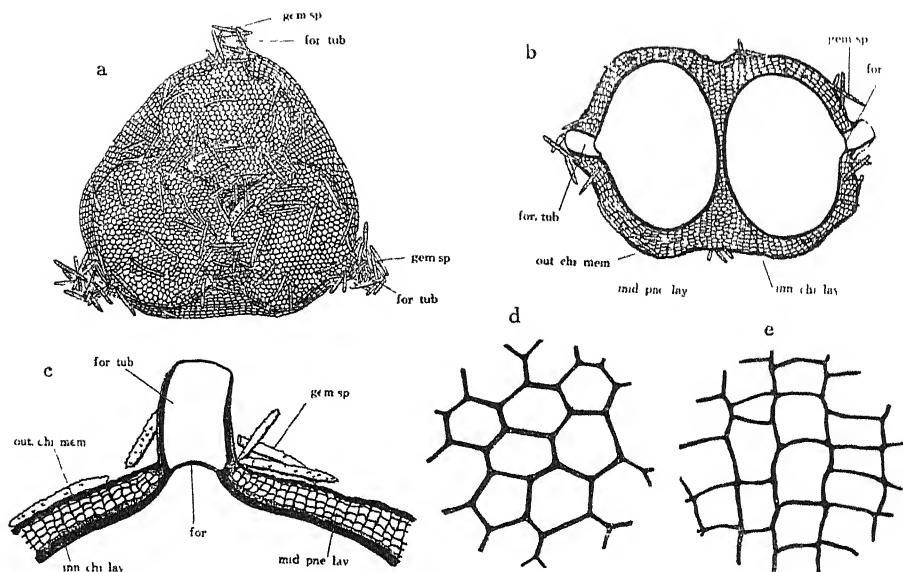
The skeleton is more compact than in the case of *Spongilla lacustris*.

The vertical fibres are well-defined, irregularly ramifying, relatively slender, usually composed of 2-5 or more spicules in cross-section, and are cemented together by a small quantity of spongin.

The transverse fibres are poorly developed, composed of 1-2 spicules in cross-section, and are always dispersed among the vertical fibres.

Gemmules (Text-fig. 5). The gemmules are produced freely among the interstices of the skeleton.

From two to eight or more gemmules are connected and are invested with a common pneumatic covering (Text-fig. 5, Figs. a, b). The size of



Text-fig. 5. *Spongilla fragilis* LEIDY.

a, Gemmules. b, Section of gemmules through the foramen. c, Sagittal-section of the foramen. d, e, A part of the pneumatic layer, cut horizontally and vertically, respectively. (a, b $\times 60$; c $\times 180$; d, e $\times 600$).

for., foramen; for. tub., foraminal tubule; gem-sp., gemmule-spicule; inn. chi. lay., inner chitinous layer, mid. pne. lay., middle pneumatic layer; out. chi. mem., outer chitinous membrane.

such structure is rather variable being measured 550–1050 μ (average 750 μ) across.

The covering is composed of a thick pneumatic layer of 15–35 μ thick.

The pneumatic layer consists of relatively large, polygonal "air-cells" arranged in several tiers (Text-fig. 5, b, c, d, e).

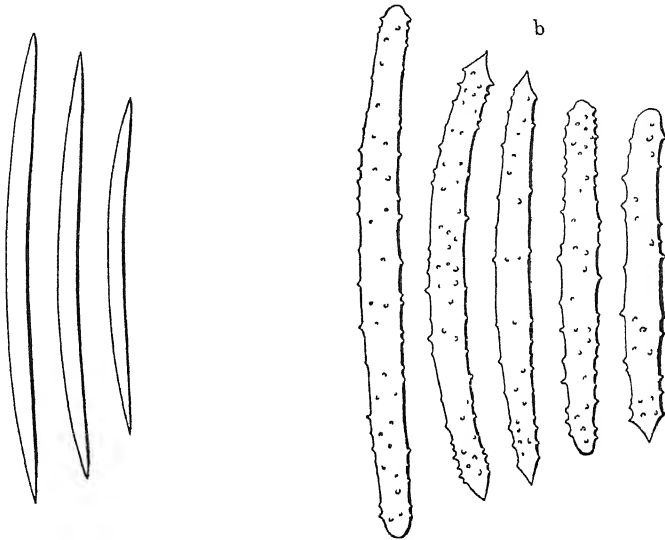
Sometimes gemmules are arranged in a single sheet located at the base of the sponge, invested with a common covering which is similar in structure with those above alluded to.

Each gemmule is spherical in shape and is rather small, varying in size, measured 280–420 μ (average 344 μ) in diameter. It is covered with a thin chitinous layer, of 4–7 μ thick. Each gemmule is yellowish or brownish in colour, with a single foramen which is provided with a straight or curved foraminal tubule (Pl. VII, Fig. 31), projecting outwards through the pneumatic covering. The foraminal tubule is 50–90 μ (average 78 μ) long and is 35–45 μ (average 40 μ) in its greatest diameter.

The gemmule spicules are found lying irregularly not only in or upon

the pneumatic covering of the gemmules, but also in the foraminal tubule.

Spicules (Text-fig. 6). The skeleton-spicules (Text-fig. 6, a) are smooth, relatively large, being $250\text{--}360\ \mu$ (average $323\ \mu$) long and $11\text{--}15\ \mu$ (average $13.4\ \mu$) thick in the thickest portion.



Text-fig. 6 *Spongilla fragilis* LEIDY.

a, Skeleton-spicules. b, Flesh-spicules. (a $\times 180$; b $\times 600$).

The flesh-spicules are lacking.

The gemmule-spicules (Text-fig. 6, b) are cylindrical or rod-shaped, often somewhat swollen in the middle, straight or feebly curved, abruptly or bluntly pointed at both ends, sometimes round-ended. They are covered with short, minute spines, and are variable in form and size, being $68\text{--}118\ \mu$ (average $92.1\ \mu$) long and $6\text{--}8\ \mu$ (average $7\ \mu$) thick in the thickest portion.

Remarks. — This species was originally described by LEIDY in 1851.

This sponge seems to be cosmopolitan and many varieties have been described by several investigators.

From Japan, ANNANDALE reported this sponge for the first time in 1909.

In 1916 ANNANDALE and KAWAMURA also reported this species from Japan.

Previously known distribution. — Widely distributed in Asia, Australia, Europe, British Isles, North America, Central America. Japan: Lake

Noziri, Lake Aoki, Lake Nakatsuna, Lake Kizaki and Lake Suwa, Province of Shinano; Tôkyô; Lake Biwa, Province of Ômi; Lake Ogura near Kyôto (ANNANDALE and KAWAMURA).

Localities. — Okadabori, a small pond near Sendai, the River Hirose and Shimekirnuma, Province of Rikuzen; Tappinuma and Hakamagata Pond, Province of Mutsu; Lake Akan, and Lake Tôro, Province of Kushiro, Hokkaidô; A small pond near Nishitappu (collected by Dr. T. HAYASHI), and a small pond near Tomakomae, Province of Iburi, Hokkaidô.

4. *Spongilla akanensis*, n. sp.

(Pl. VI, Figs. 16, 17; Text-fig 7)

This species usually coats or surrounds the stems of some slender but rather hard water-weeds, grown in slowly running water at a depth of about 5 metres.

The Lake Akan where I have obtained this sponge is famous on account of the fact that there an alga *Aegagropila Sauteri* (NEES) KÛTZ are found very abundantly.

The external form of this sponge (Pl. VI, Figs. 16, 17) is that of an encrusting layer from which a number of short irregular tubercles or projections usually arise and moreover they form in most cases a very irregular network or a ramifying mass.

This sponge is relatively hard but brittle in consistency.

The colour of this sponge in life is green when grown in a good light, but when it is preserved in alcohol the green colour turns white.

The pores and oscula are great in number, but they are never large or very conspicuous.

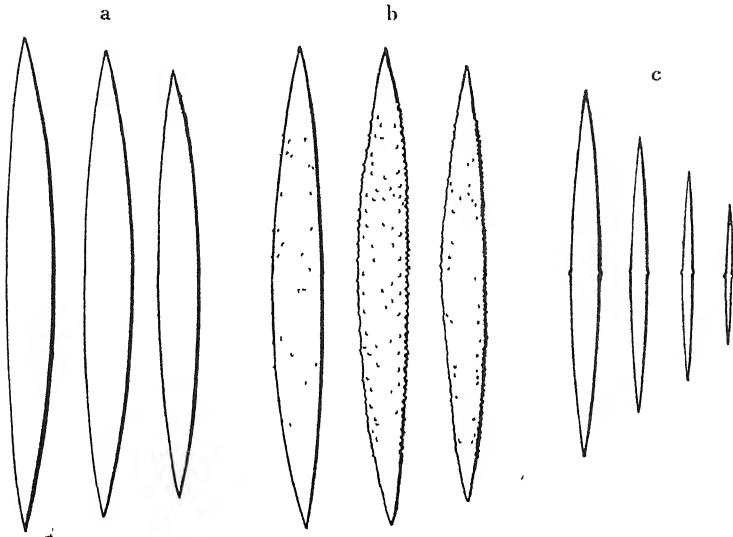
The skeleton is very compact and forms an irregular network.

Sometimes the vertical fibres show an arrangement rather well-defined, but usually they are arranged very irregularly. Each vertical fibre is composed of 2-6 or more spicules in cross-section.

The transverse fibres, each of which is composed usually of 1-2 spicules in cross-section, show a very irregular arrangement and run in every direction among the vertical fibres.

Generally the distal ends of the vertical fibres project outwards from the sponge surface to some extent and thus they are rough to touch.

Spicules (Text-fig. 7). The skeleton-spicules (Text-fig. 7, a, b) are very thick, gradually or abruptly pointed at the extremities and the rest smooth or covered with very minute spines. They are 310-360 μ (average 337 μ) long and 20-30 μ (average 25.9 μ) thick in the thickest portion.



Text-fig. 7. *Spongilla akanensis*, n. sp
 a, Smooth skeleton-spicules. b, Micro-spined skeleton-spicules.
 c, Young immature skeleton-spicules. (all $\times 180$)

There exist no true flesh-spicules; but young immature skeleton-spicules (Text-fig. 7, c) are frequently to be found, and they are $100\text{--}280\ \mu$ long and $5\text{--}25\ \mu$ thick in the thickest portion.

The gemmule-spicules are unknown to me, as I was not able to find even a single gemmule in this specimen.

Remarks. — Though I could not find even a single gemmule of this sponge it seems to be one of the most remarkable fresh-water sponges found in Japan, judging from the following features: First, this species seems to have the hardest consistence of all Japanese fresh-water sponges hitherto known; Secondly, this species has very big skeleton-spicules, which show the greatest diameter among the same kind of spicules of other species of Japanese fresh-water sponges.

On account of the absence of gemmules in this specimen, I regret that I can not determine the genus to which this sponge belongs. Judging from the characters in general it seems to be rather reasonable at present to assign this species to the genus *Spongilla*.

Locality. — Lake Akan, Province of Kushiro, Hokkaidô.

5. *Ephydatia fluviatilis* (LINNÉ)

(Pl. VI, Fig. 18, Pl. VII, Fig. 32, Text-figs. 8, 9)

Spongia fluviatilis, LINNÉ 1759, p. 1348.*Spongia canalium*, SCHRÖTER 1788, p. 149.*Spongilla fluviatilis*, LIEBERKÜHN 1856, p. 496.*Ephydatia fluviatilis*, GRAY 1867, p. 550.*Spongilla sceptrifera*, BOWERBANK 1874, p. 300.*Meyenia fluviatilis*, CARTER 1881, p. 92; POTTS 1887, p. 219.*Meyenia* No. 1, DYBOWSKY 1882, p. 13.*Meyenia angustibiotulata*, CARTER 1885, p. 454.

This sponge usually grows on the surface of logs, branches, roots, stones and sometimes at the sandy bottom of quiet or flowing water at a depth of 1–10 metres or more.

The external form of this sponge (Pl. VI, Fig. 18) is that of an encrusting layer provided with a number of irregular, lobular projections of various sizes.

Occasionally it is irregularly massive with surface very even.

The sponge is either hard or soft, and comparatively fragile in consistence.

The colour of the sponge in life is green when it is grown in the light, but it is often yellow, cream-coloured, white, grey or brown when grown in the shade.

Its pores and oscula are generally inconspicuous but in some specimens they grow large and are prominent with border circular.

The skeleton fibres are well-defined, rather compactly woven, and thus form a very irregular network.

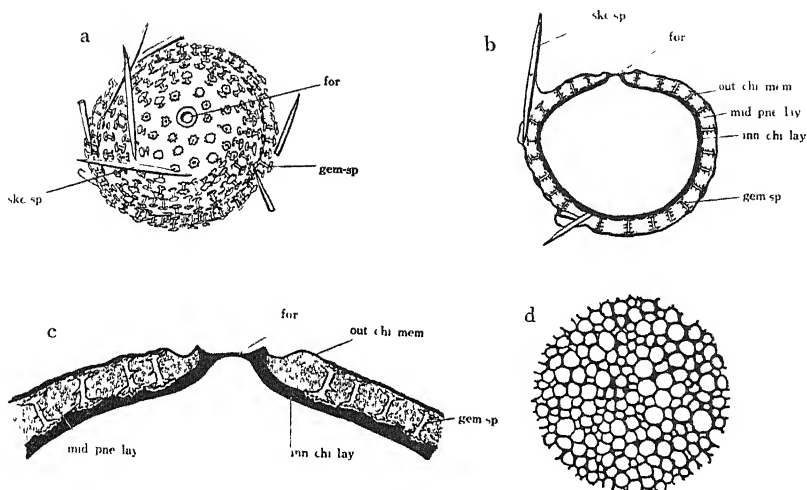
The vertical fibres which are composed of 3–10 spicules in cross-section, run nearly in all directions ramifying with one another.

The transverse fibres which are composed of 1–3 spicules in cross-section are rather poorly developed and are distributed among the vertical fibres without any definite orientation.

Gemmules (Text-fig. 8). The gemmules are produced freely and abundantly among the interstices of the skeleton forming the base of the sponge.

They are spherical in form (Text-fig. 8, a), yellow or brown in colour, and are of relatively small size, measuring $40\text{--}47\ \mu$ (average $45\ \mu$) in the greatest diameter.

They are usually covered with a rather thick pneumatic coat in which the gemmule-spicules are arranged radially (Text-fig. 8, b, c).

Text-fig. 8. *Ephydatia fluviatilis* (L.)

a, Gemmule, its foramen is shown in the center. b, Section of the gemmule through the foramen. c, Sagittal-section of the foramen. d, A part of the pneumatic layer (a, b $\times 60$, c $\times 180$; d $\times 600$). for, foramen; gem-sp., gemmule-spicule, inn. chi. lay., inner chitinous layer; mid. pne. lay., middle pneumatic layer; out. chi. mem., outer chitinous membrane; ske-sp., skeleton-spicule.

The pneumatic coat is, as a rule, composed of the following three layers, viz. 1) the inner thick chitinous layer which is $8-10\ \mu$ in thickness; 2) the middle pneumatic layer which is composed of granular "air-cells" (Text-fig. 8, d) is $25-35\ \mu$ thick; and 3) the outer chitinous membrane of $2-5\ \mu$ thickness.

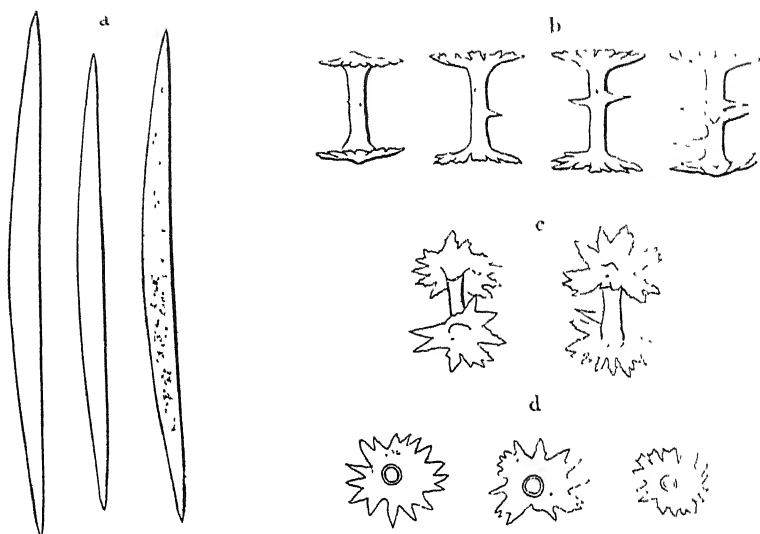
Each gemmule has commonly a single foramen (Text-fig. 8, a, b, c; Pl. VII, Fig. 32) which is not protected by any distinct cup-like or tubular structures, except for the thin chitinous operculum attached to the orifice.

The diameter of the foramen is $40-55\ \mu$ and the diameter of the chitinous operculum is $20-25\ \mu$.

Spicules (Text-fig. 9). The skeleton-spicules (Text-fig. 9, a) are relatively large and are straight or slightly curved, gradually and sharply pointed. They are smooth or covered with very minute spines and are $300-400\ \mu$ (average $350\ \mu$) long and $15-24\ \mu$ (average $19.3\ \mu$) thick in the thickest portion.

There are no flesh-spicules.

The gemmule-spicules (Text-fig. 9, b, c, d) are amphidiscs or birotulates, and are small, with surface smooth or covered with very minute spines.

Text-fig. 9. *Ephydatia fluviatilis* (L.)

a, Skeleton-spicules. b, Gemmule-spicules (viz. amphidiscs or birotulates), side view. c, Oblique view of the same. d, Apical view of the rotules of the same. (a $\times 180$, b, c, d $\times 600$).

The length of the shaft is always longer than the diameter of the rotule. The shaft is usually straight, smooth or occasionally spinous, $21\text{--}29\ \mu$ (average $25.6\ \mu$) long and $3\text{--}4\ \mu$ (average $3.4\ \mu$) in diameter. The spines on the shaft, 1–7 in number, are relatively large, straight, stout and tapering.

The rotules are in general flat, moderately serrated in margin and are sometimes covered with a few minute spines. They are $18\text{--}23\ \mu$ (average $21.1\ \mu$) in diameter.

Remarks.—This species is also one of the most widely distributed fresh-water sponges, and we may find many varieties and synonyms described by various authors.

This sponge was originally described in 1759 by LINNÉ in the name of *Spongia fluviatilis* in the 10th edition of *Systema Naturae*.

From Japan, this species was first reported in 1895 by D. W. WELTNER; the specimens on which his description was based were those secured by HILGENDORF in Tôkyô in 1882.

In 1933, I was able to obtain this species from Lake Abashiri in Hokkaidô.

Previously known distribution.—Widely distributed in Asia, Australia,

Europe, British Isles, Africa, North America. Japan; Tôkyô (HILGENDORF).

Locality. — Lake Abashiri, Province of Kitami, Hokkaidô.

6. *Ephydatia mulleri* (LIEBERKÜHN)

(Pl. VI, Figs 19, 20, 21, 22, 23, 24, Pl VII. Fig 33, Text-figs 10, 11).

Spongilla mülleri, LIEBERKÜHN 1856, p. 510.

Trachyspongilla mülleri, DYBOWSKY 1878, p. 53.

Spongilla astrosperma, POTTS 1880, p. 357.

Meyenia No. 2, No. 3 α , β , DYBOWSKY 1882, pp. 15–19.

Spongilla mirabilis, RETZER 1883, p. 25.

This sponge grows on the surface of the submerged logs, trunks, branches, rocks and stones in quiet as well as rapidly running water at a depth of 1–5 metres.

The external form of this species is that of a mass or rather flat crust, from which sometimes a number of stout finger-like projections arise (Text-figs. 19–24). Its surface is usually uneven and is rugged or knobbed.

The consistence of this sponge is generally soft and fragile.

Its colour is green when it is grown in sufficient sun-light, but is yellow, white or grey in faint light or in shade.

The oscula are relatively large and the pores are small and numerous.

The vesicular cells or “bubble-cells” are always found abundantly in the parenchyma.

The skeleton is rather compact. The vertical fibres which are composed of 3–8 or more spicules in cross-section, are well-defined but are irregularly ramified; while the transverse fibres, each of which commonly consists of 1–3 spicules in cross-section, are rather poorly developed.

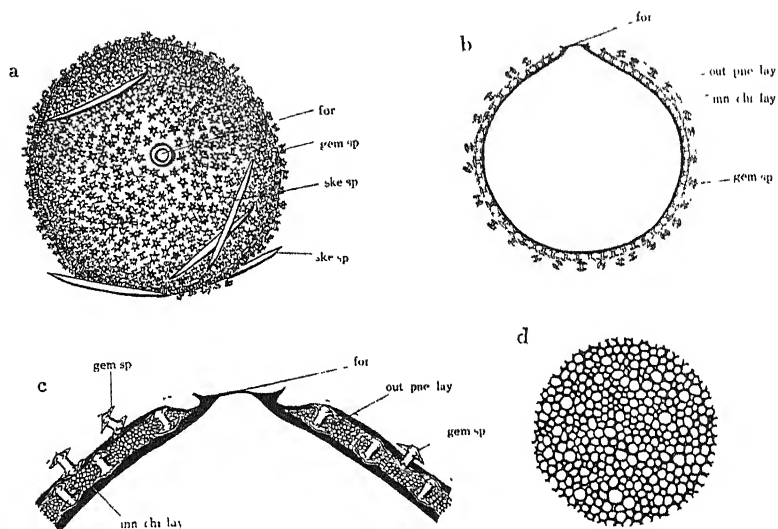
The transverse fibres occur fairly thickly in the gemmule-region.

Gemmules (Text-fig. 10). The gemmules are produced in the interstices of the skeleton fibres and are found especially abundant at the base of the sponge body.

The gemmules are very variable in both shape and size. They are usually spherical (Text-fig. 10, a) but sometimes are ellipsoidal or ovoidal in form, measured 250–770 μ (average 557 μ) in the greatest diameter.

They are yellow or brown in colour.

Each gemmule is covered with a pneumatic coat (Text-fig. 10, b, c) composed of the inner chitinous layer and of the outer pneumatic layer. The inner chitinous layer is relatively thin and is 3–5 μ thick. In this inner chitinous layer, one of the rotules of each birotulate is usually imbedded.



Text-fig. 10 *Ephydotia mulleri* (LIEBERKÜHN)

a, Gemmule, the foramen is shown in the center. b, Section of a gemmule through the foramen. c, Sagittal section of the foramen. d, A part of the pneumatic layer. (a, b $\times 60$, c $\times 180$, d $\times 600$).
 for, foramen, gem-sp., gemmule spicule; inn. chi lay, inner chitinous layer; out. pne. lay., outer pneumatic layer; ske-sp., skeleton-spicule.

The outer pneumatic layer is composed of numerous small granular "air-cells" (Text-fig. 10, d) and is about 15μ thick. In this layer, the birotulates are found, being arranged radially. This layer is sometimes covered with a poorly-developed, half-chitinized layer of $2\text{--}3\mu$ thick.

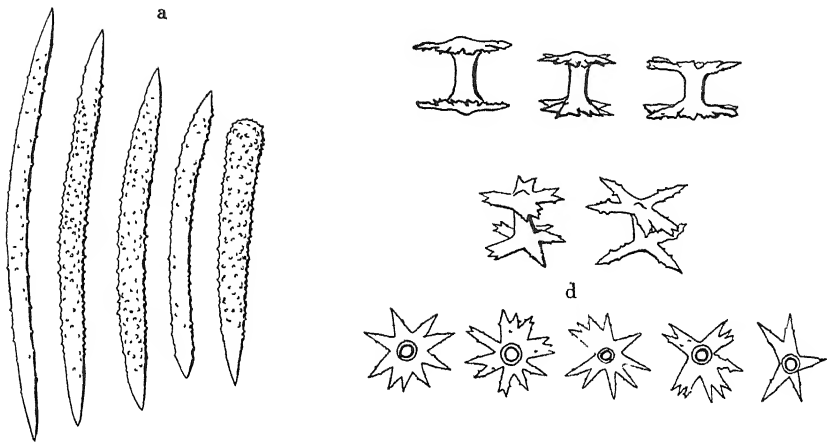
Ordinarily the gemmule-spicules are arranged in a single row in the pneumatic layer, but sometimes, one or more rows of these spicules in an irregular disposition may be added (Text-fig. 10, b).

Generally each gemmule has a single foramen (Pl. VII, Fig. 33), the shape of which is like a shallow dish. The diameter of the foramen (out-side) is $35\text{--}80\mu$ and the same of the inner operculum is $20\text{--}30\mu$.

Spicules (Text-fig. 11). The skeleton-spicules (Text-fig. 11, a) are rather stout, straight or slightly curved, sharply or abruptly pointed at the extremities and, as a rule, they are densely covered with minute spines, except for their two ends, $230\text{--}310\mu$ (average 270μ) long and $10\text{--}20\mu$ (average 15.8μ) thick in the thickest portion.

There are no flesh-spicules.

The gemmule-spicules (Text-fig. 11, b, c, d) are small amphidiscs or birotulates.

Text fig 11. *Ephydatia mulleri* (LIEBERKÜHN).

a, Skeleton-spicules. b, Gemmule-spicules, side-view c, Oblique view of the same. d, Apical view of the rotules of the same. (a $\times 180$; b, c, d $\times 600$).

The shaft of the birotulate is usually shorter or rarely longer than the diameter of the rotule. It is short, stout, straight and is usually smooth, $13\text{--}18\ \mu$ (average $15.7\ \mu$) long and $4\ \mu$ in diameter. The rotule is flat and its margin is cut deeply and irregularly as to form 5–10 or more teeth.

Sometimes it is covered with scattered micro-spines. The rotule is measured $18\text{--}25\ \mu$ (average $21.5\ \mu$) in diameter.

Remarks.—This sponge was first described in 1856 by LIEBERKÜHN by the name of *Spongilla mülleri*.

From Japan this species was reported for the first time by WELTNER the specimen being obtained from Tôkyô. In 1909, ANNANDALE reported that this sponge was collected by Dr. OKA at Kameido near Tôkyô in October of 1901.

This species is also one of the fresh-water sponges that have a very wide distribution.

Fortunately in 1933 I could find this species growing luxuriantly in Lake Shikaribetsu, Hokkaidô.

Previously known distribution.—Asia, Europe, British Isles, North America. Japan: Kameido, near Tôkyô (collected by Dr. OKA).

Localities.—Lake Biwa, Province of Ômi; Utorinuma (collected by Dr. HÔZAWA and Mr. K. Irô) and Kuwanuma, Province of Rikuzen; A canal of Ôyô Park in Hirosaki, Province of Mutsu; Lake Abashiri, Province of Kitami, Hokkaidô; Lake Shikaribetsu, Province of Tokachi, Hokkaidô; Utonaitonuma, Province of Iburi, Hokkaidô; Tokotan-numa

near Akkeshi, Province of Kushiro, Hokkaidô (collected by Mr. Y. HADA).

7. *Ephydatia mülleri* var. *japonica* (HILGENDORF)

(Pl. VI, Figs 25, 26, Pl VII, Fig 34, Text-figs. 12, 13)

Spongilla fluviatilis var. *japonica*, HILGENDORF 1882, p. 26.

Ephydatia fluviatilis var. *japonica*, WELTNER 1895, pp. 123, 134.

Ephydatia japonica, ANNANDALE 1909, pp. 109-110.

Ephydatia mülleri var. *japonica*, ANNANDALE and KAWAMURA 1916, p. 13.

This sponge usually grows upon the surface of submerged bodies such as logs, branches, twigs, stems of reeds, roots, rocks, stones, pillars of bridges, stone-walls, and sometimes at sandy or muddy bottoms. It is found in both quiet and rapidly running waters at a depth of 0.5-5 metres.

The external form of this sponge (Pl. VI, Figs. 25, 26) is generally that of a flat layer from which sometimes long or short, cylindrical projections arise.

But, according to the circumstances, especially in quiet water, it is frequently massive. In one of the largest specimens at hand the length, breadth and height measured 10, 8 and 7 cm., respectively.

Its surface is usually uneven, being rugged and rough.

The sponge is rather soft and fragile in consistence.

Its colour is usually green when the sponge is exposed in a good light, but it may become yellow, grey or black according to the environmental conditions.

The oscula are relatively large and deep, but in some specimens they are small and obscure. The pores are usually numerous but small.

The skeleton fibres are rather well-defined, especially in the gemmule region, but they are relatively slender forming an irregular network.

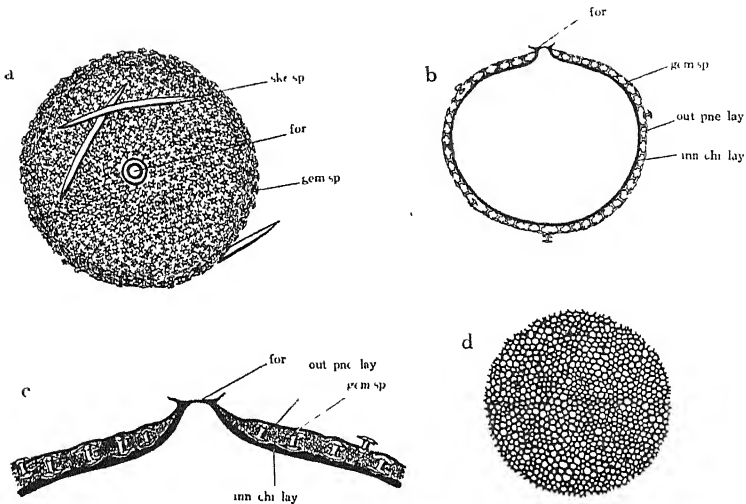
The vertical fibres, each of which is composed of 3-8 or more spicules in cross-section, run vertically towards the surface and are irregularly branched.

The transverse fibres each of which is composed of usually 1-2 spicules, are dispersed among the vertical fibres.

Gemmules (Text-fig. 12). The gemmules are numerous found in the interstices of the skeleton, and are especially abundant in the lower part of the sponge.

Though they vary to some extent both in form and size, the gemmules are mostly spherical (Text-fig. 12, a) and measure 400-650 μ (average 505 μ) in diameter. Their colour is white, yellow or brown.

The gemmule is covered with a pneumatic coat (Text-fig. 12, b, c).



Text-fig. 12. *Ephedratia mulleri* var *japonica* (HILGENDORF).

a, Gemmule, showing a foramen in the centre. b, Section of a gemmule through the foramen c, Sagittal section of the foramen d, A part of the pneumatic layer (a, b $\times 60$; c $\times 180$; d $\times 600$).

for, foramen; gem-sp., gemmule-spicule, inn chi. lay., inner chitinous layer, out pne. lay., outer pneumatic layer, ske-sp, skeleton-spicule

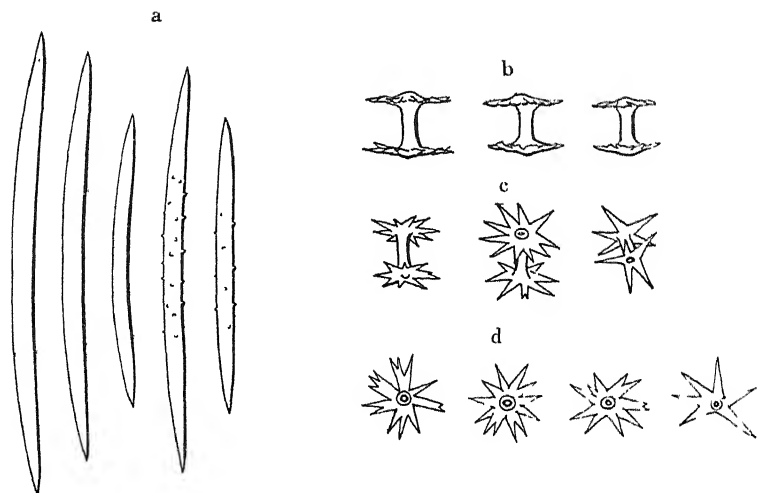
In this coat the birotulates are in most cases radially arranged forming a single row but sometimes one or more rows of the same kind of spicules may be added to the above. In such case the birotulates are arranged either radially or irregularly. The pneumatic coat is composed of the inner chitinous layer 4–5 μ thick and the outer pneumatic layer of 13–15 μ thick.

The pneumatic layer which consists of very small granular “air-cells” (Text-fig. 12, d), is sometimes covered externally by a thin half-chitinized layer. As a rule, each gemmule has a single foramen (Pl. VII, Fig. 34) the shape of which is like a shallow dish. The diameter of the foramen is 42–70 μ and the same of the operculum is 15–17 μ .

Spicules (Text-fig. 13). The skeleton-spicules (Text-fig. 13, a) are rather slender, straight or slightly curved, gradually and sharply pointed with surface usually smooth or sometimes with a few scattered minute spines in their middle parts, 220–360 μ (average 283 μ) long and 10–16 μ (average 12.9 μ) thick in the thickest portion.

There are no flesh-spicules.

The gemmule-spicules (Text-fig. 13, b, c, d), which are birotulates or amphidiscs are very small and their surface is smooth.



Text-fig 13. *Ephydatia mulleri* var. *japonica* (HILGENDORF).
 a, Skeleton-spicules. b, Gemmule-spicules, side view. c, Oblique view
 of the same. d, Apical view of the rotules of the same. (a $\times 180$;
 b, c, d $\times 600$).

Their shafts are generally shorter than the diameter of the rotules and are smooth and straight, being $12-15\ \mu$ (average $14\ \mu$) long and $2.5-3.5\ \mu$ (average $3\ \mu$) thick.

The rotules are generally flat with surface entirely smooth but their margin is deeply serrated, forming usually 5-12 teeth. The rotules are measured $16-21\ \mu$ (average $18.4\ \mu$) in diameter.

Remarks.—This sponge was originally described by HILGENDORF as *Spongilla fluviatilis* var. *japonica* in 1882, its description being based upon the specimen obtained in Tôkyô.

This variety differs from the typical *Ephydatia mülleri* only in having skeleton-spicules that are as a rule very smooth but sometimes bear a few minute scattered spines in the central region.

This sponge is found very commonly being widely distributed in the Main Island of Japan.

Previously known distribution.—North America, Manchuria. Japan: Chôsen; Yanaidzu, Province of Rikuzen; Lake Aoki, Lake Nakatsuna, Lake Kizaki and Lake Suwa, Province of Shinano; Lake Biwa, Province of Ômi; A small pond at Yodo, and Ôsawa Pond near Kyôto, Province of Yamashiro; Okayama, Province of Bizen and Hiroshima, Province of Aki (ANNANDALE and KAWAMURA).

Localities.—Suwanuma (collected by Mr. K. Itô) near Sanuma, several

ponds in Sendai and the River Hirose, Province of Rikuzen; A canal of Ôyô Park in Hirosaki, Province of Mutsu; Lake Akan, Province of Kushiro, Hokkaidô; Lake Abashiri, Province of Kitami, Hokkaidô.

8. *Heteromeyenya baileyi* var. *petri* (LAUTERBORN)

(Pl VI, Figs 27, 28; Pl VII, Fig. 35, Text-figs 14, 15).

Carterius stepanowi forma *petri*, LAUTERBORN 1902, p. 528.

Heteromeyenya kawamurae, ANNANDALE 1916, pp. 14-15.

Heteromeyenya baileyi var. *petri*, SCHRÖDER 1927, p. 107.

The sponge is found in quiet or slowly running water, usually encrusting the submerged logs, branches, twigs, roots and stones at a depth of a few metres.

The external form of this sponge (Pl. VI, Figs. 27, 28), as a rule, is that of a thin film with a number of short anastomosing branches, but sometimes it may be irregularly massive.

The sponge is very soft and fragile in consistence.

The colour of this sponge in life is usually bright green; but it will turn yellow, white or brown, being affected by the environmental conditions.

The skeleton fibres which are mainly composed of vertical fibres, are well-defined but they are relatively slender, each fibre being composed of 2-5 or more spicules in cross-section.

These fibres ramify very loosely in the larger parts of the sponge body but in the gemmule region they ramify rather densely and form an irregular network.

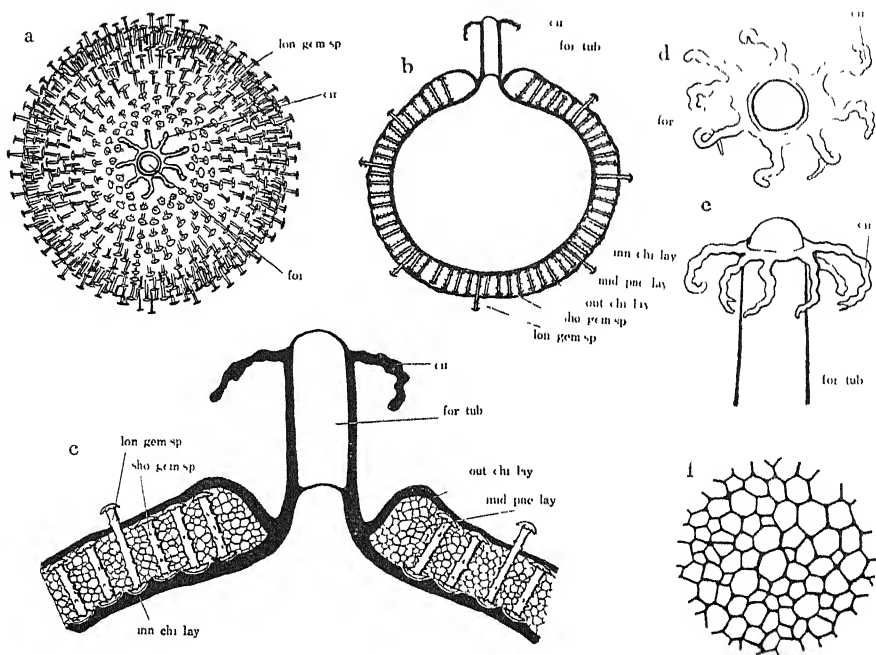
The distal ends of vertical fibres freely project beyond the dermal membrane and thus make the sponge surface hairy.

The transverse fibres are poorly developed and they are either connected with vertical fibres or are freely distributed among the vertical fibres.

Gemmules (Text-fig. 14). The gemmules are formed abundantly and freely in the interstices of the skeleton.

They are usually spherical in form (Text-fig. 14, a), white or yellow in colour and are of moderate size, measuring 530-700 μ (average 611 μ) in the greatest diameter. Each gemmule is covered with a pneumatic coat (Text-fig. 14, b, c) in which the gemmule-spicules are arranged radially.

The coat is composed of three layers; viz. 1) the inner chitinous layer, of 6-8 μ thick; 2) the middle pneumatic layer which consists of relatively small "air-cells" (Text-fig. 14, c, f), and which is 40-55 μ thick; and 3) the outer chitinous layer of 3-5 μ thick.



Text-fig. 14. *Heteromeyenia baileyi* var. *petri* (LAUTERBORN).

a, Gemmule, its foramen is shown in the center. b, Section of a gemmule through the foramen. c, Sagittal-section of the foramen. d, Apical view of the foramen with cirrus appendages. e, Side view of the same. f, A part of the pneumatic layer. (a, b $\times 60$; c, d, e $\times 180$, f $\times 600$).

cir., cirrus appendages; for. tub, foraminal tubule, inn. chu. lay., inner chitinous layer, lon. gem-sp., longer gemmule-spicule; mid. pne. lay., middle pneumatic layer, sho. gem-sp., shorter gemmule-spicule, out. chu. lay., outer chitinous layer

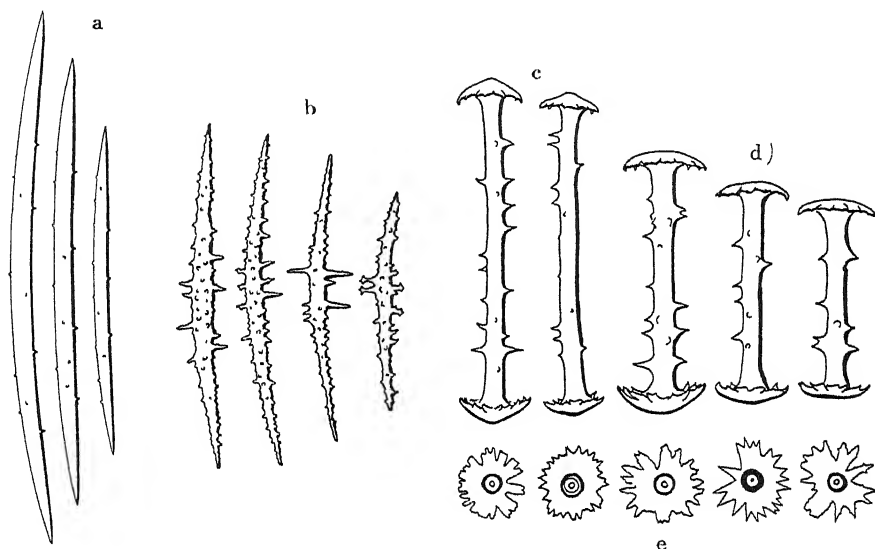
There exist two types of gemmule-spicules, namely the longer and the shorter. The shorter ones lie between the outer and inner chitinous layers taking radial arrangement. The longer spicules are so placed that one part of their shaft together with their distal rotule will project freely beyond the outer chitinous layer of the gemmule.

Each gemmule has a single, characteristic foramen (Pl. VII, Fig. 35, Text-fig. 14, d, e) which is provided with a long, stout, straight or slightly curved tubule, with cirrus appendages attached to its distal end.

The length of the foraminal tubule measured from the inner chitinous operculum to its tip, is $100\text{--}125\mu$ and its diameter is $35\text{--}45\mu$ at the thickest portion. The diameter of the chitinous operculum is $27\text{--}30\mu$. The appendages of the foraminal tubule are usually 6–10 in number.

They are transparent, and are somewhat irregularly crooked and hang downwards. They are variable in shape and size, measuring $45\text{--}75\ \mu$ long and $5\text{--}10\ \mu$ thick.

Spicules (Text-fig. 15). The skeleton-spicules (Text-fig. 15, a) of moderate size are relatively slender, straight or slightly curved, sharply and gradually pointed at the extremities, nearly smooth or covered with a few minute spines, $240\text{--}400\ \mu$ (average $325\ \mu$) long and $10\text{--}14\ \mu$ (average $12.4\ \mu$) thick in the thickest portion.



Text-fig. 15. *Heteromeyema baileyi* var. *petri* (LAUTERBORN).

a, Skeleton-spicules b, Flesh-spicules. c, Longer gemmule-spicules. d, Shorter gemmule-spicules. e, Apical view of the rotules of the gemmule-spicules. (a $\times 180$; b, c, d, e $\times 600$).

The flesh-spicules (Text-fig. 15, b) are small, slightly curved, gradually pointed at both ends and are covered with long and short spines of variable size.

They are $61\text{--}72\ \mu$ (average $69.5\ \mu$) long and $3\text{--}4\ \mu$ (average $3.2\ \mu$) thick in the thickest portion.

The gemmule-spicules may be classified into two kinds (Text-fig. 15, c, d), the longer and the shorter, but their difference in length and thickness is not very distinct being obliterated by the existence of intermediate forms.

The shaft of the shorter spicule is $4\ \mu$ thick and is slightly stouter than that of the longer which measures $3.5\ \mu$ thick. In number the

shorter is superior to the longer. In both kinds of spicule the shaft of the birotulate is straight or sometimes slightly curved, rather slender and either smooth or armed with a few scattered spines of relatively large size.

The rotules (Text-fig. 15, e) are rather flat but sometimes are feebly incurved. The margin of the rotule is, as a rule, deeply and irregularly serrated and the teeth thus formed are angular and rarely curved.

The rotule of the longer gemmule-spicule measured $15-17\mu$ (average 16.2μ) in diameter, and is slightly smaller than that of the shorter which is $18-20\mu$ (average 18.7μ) in diameter. The length of the longer gemmule-spicule is $70-76\mu$ (average 72.4μ) and the same of the shorter is $45-56\mu$ (average 49.6μ).

Remarks.—This species was first described by LAUTERBORN in the name of *Carterius stepanowi* in 1902.

In 1916 Dr. N. ANNANDALE and Prof. T. KAWAMURA reported this form from Japan in the name of *Heteromeyenya kawamurae*. In the description they have given the existence of the cirrus appendages of the foraminal tubule was missed.

In 1927, in examining the specimens from Japan, Dr. SCHRÖDER concluded that the sponge which was described by ANNANDALE and KAWAMURA as *Heteromeyenya kawamurae* is identical with *Heteromeyenya baileyi* var. *petri* of LAUTERBORN.

Previously known distribution.—Germany; Czecho-Slovakia; Chili. Japan: Lake Biwa, Province of Ômi; The River Tenryû, Province of Shinano (ANNANDALE and KAWAMURA).

Localities.—Lake Ogura near Kyôto, Province of Yamashiro; Futatsusawa near Sendai (collected by Mr. K. Itô), Suwanuma near Sanuma (collected by Mr. K. Itô), Province of Rikuzen; Lake Abashiri, Province of Kitami, Hokkaidô.

Key to the fresh-water sponges of Hokkaidô.

- A. Gemmule-spicules rod-shaped, straight or curved, spined, without transverse discs at the extremities, (Genus *Spongilla*).
 - (I) Flesh-spicules present; pointed, spined acerates.
 1. Skeleton-spicules numerous, flesh-spicules rather few, *S. lacustris*.
 2. Skeleton-spicules few, flesh-spicules very numerous, *S. shikarbensis*
 - (II) Flesh-spicules absent.
 1. Sponge soft; gemmules covered with coating of air-cells resembling a honey-

- comb, *S. fragilis*.
- 2 Sponge very hard, skeleton-spicules big, micro-spined,
 *S. akanensis*.
- B. Gemmule-spicules with transverse discs at the extremities.
- (I) Birotulates of one kind; flesh-spicules absent (Genus *Ephydatia*).
- a. Shaft of birotulates longer than rotule diameter, no vesicular cells, .
 *E. fluviatilis*.
- b. Shaft of birotulates as long as or shorter than rotule diameter; vesicular cells present.
- 1 Skeleton-spicules micro-spined, *E. mulleri*.
- 2 Skeleton-spicules smooth or nearly so, *E. mulleri* var. *japonica*.
- (II) Birotulates of two kinds, one considerably longer than the other.
 (Genus *Heteromeyenia*).
- Flesh-spicules present, spined acerates, *H. baileyi* var. *petri*

LIST OF REFERENCES

- ANNANDALE, N. (1909) Report on a collection of freshwater sponges from Japan Annot. Zool. Japonenses, Vol. 7, pp. 105-112.
- and KAWAMURA, T. (1916). The Sponges of Lake Biwa Journ. Coll. Sci. Imp. Univ. Tokyo, Vol. 39, Art. 1, pp. 1-27.
- ARNDT, W., (1926). Die Spongillidenfauna Europas Archv. f. Hydrobiologie, Vol. 17, pp 337-365
- (1928) Porifera, Schwämme, Spongien. Die Tierwelt Deutschlands, Jena. Teil 4, pp 1-94
- BOWERBANK, I S., (1863). A monograph of the Spongillidae. Proc. Zool. Soc London, pp 440-472.
- (1874) A Monograph of the British Spongiadae. London, Vol 3, p 300
- BROTHERUS, A H. (1876) Om slägtet Spongilla, Helsingfors, p. 13
- CARTER, H. J., (1881). History and Classification of the known species of Spongilla Ann. Mag Nat. Hist (ser. 5) Vol. 7, pp. 77-107
- (1885). On a variety of the Freshwater Sponge *Meyenia fluviatilis* Ann. Mag Nat. Hist., (ser 5) Vol. 15, p. 453
- DYBOWSKI, W. (1878) Mitteilungen über Spongien II Zool. Anz. Vol. 1, p 53.
- (1882) Studien über die Süsswasser-Schwämme des russischen Reiches. Mem Acad. Sci. St Petersburg. (ser. 7) Vol. 30, pp. 13-19.
- EIHRENBURG, C. G. (1841). Weitere Resultate über Untersuchungen über die in Berlin lebenden mikroskopischen unterirdischen Organismen. Mon. Ber. Kgl. Akad. Wiss. Berlin, p. 363.
- ESPER, E I. C. (1794). Die Pflanzenthier. Teil 2, p. 235.
- GEE, N. GIST, (1928) Note on Oriental Fresh-water Sponges, II. Lingnan Sci Journ. Vol. 6, No. 3, pp 221-225.
- (1930-1931). A contribution toward an alphabetical list of the Known Fresh water Sponges. Peking Nat. Hist. Bulletin, Vol. 5, pp. 31-52.
- and WU C. F. (1927). Chinese Fresh Water Sponges. Bull. Peking Soc. Nat. Hist., Vol. 2, Partl, pp. 1-14.
- GRAY, I. E., (1867). Notes on the Arrangement of Sponges with the Description of some New Genera Proc. Zool. Soc. London, p. 550.

- HILGENDORF, F., (1882). *Spongilla fluviatilis* LIEBERKÜHN var *japonica* Sitz. Ber. Ges. Naturf. Freund. p. 26.
- KAWAMURA, T., (1916). A Key to the Fresh-water Sponges of Japan and their Distribution Dobutsugaku Zasshi, Vol. 28, pp. 10-11.
- LAMARCK, I. B. P. A. de M., (1816). Histoire des Animaux sans Vertèbres. Paris, Vol. 2, p. 100.
- LAUTERBORN, R., (1902). Ein für Deutschland neuer Süßwasserschwamm (*Carterius stepanowi* DYB.) Biol. Zentralbl., Vol. 22, p. 519.
- LEIDY, I., (1851). *Spongilla fragilis*. Proc. Acad. Nat. Sci. Philadelphia, p. 278.
- LIEBERKÜHN, N., (1856). Zusätze zur Entwicklungsgeschichte der Spongillen. Arch. Anat. Phys., pp. 496-510.
- LINNÉ, C. v., (1759). Systema Naturae. Holmiae, 10th edition, Vol. 2, p. 1348.
- NOLL, F. C., (1870). Flusssquarien, Zool. Garten, Vol. II, p. 173.
- (1880). *Spongilla glomerata* NOLL Zool. Anzeiger, Vol. 9, p. 682.
- OLD, M. C., (1931). Taxonomy and Distribution of the Fresh-water Sponges (Spongillidae) of Michigan. Papers Mich. Acad. Sci., Arts and Letters, Vol. 15, pp. 439-477.
- POTTS, E., (1880). Fresh-water sponges of Fairmount Park Proc. Acad. Nat. Sci., Philadelphia, p. 330.
- (1887). Contributions towards a Synopsis of the American Forms of Fresh water Sponges with Descriptions of those named by other Authors and from all parts of the World. Proc. Acad. Nat. Sci., Philadelphia, pp. 158-296.
- RETZER, W., (1883). Die deutschen Süßwasserschwämme, Tübingen, p. 25.
- SHRÖDER, K., (1927). Über die Gattungen *Carterius* PETR., *Astromeyenia* ANNANDALE und *Heteromeyenia* POTTS (Porifera. Spongillidae) Spongilliden-Studien III. Zool. Anz., Vol. 73, pp. 101-112.
- SCHRÖTER, I. S., (1788). Beschreibung einer neuen Spongie der süßen Wasser. Spongia canalium. Der Naturforscher, St., Vol. 23, p. 149.
- THIENEMANN, F. A. L. W., (1828). Lehrbuch der Zoologie. In: NAUMANN, L. F., REICHENBACH, H. L. und THIENEMANN, F. A. L. W., Encyclopädie der speziellen Naturgeschichte, Vol. 3, Berlin.
- WELTNER, W., (1895). Spongillidenstudien III, Katalog und Verbreitung der bekannten Süßwasserschwämme Arch. f. Natg., Vol. 1, pp. 123-131.

EXPLANATION OF THE PLATES

PLATE IV.

- Fig. 1 *Spongilla lacustris* (L.); $\times 3/14$. Lake Akan.
- Fig. 2 *Spongilla lacustris* (L.); $\times 9/16$. Lake Akan.
- Fig. 3 *Spongilla lacustris* (L.); $\times 3/4$. Lake Akan.
- Fig. 4 *Spongilla lacustris* (L.); $\times 3/4$. Lake Akan.
- Fig. 5 *Spongilla lacustris* (L.); $\times 1/4$. Lake Akan.
- Fig. 6 *Spongilla lacustris* (L.); $\times 3/4$. Lake Akan.
- Fig. 7 *Spongilla lacustris* (L.); $\times 3/4$. Lake Akan.
- Fig. 8 *Spongilla lacustris* (L.); $\times 3/4$. Lake Abashiri.

PLATE V.

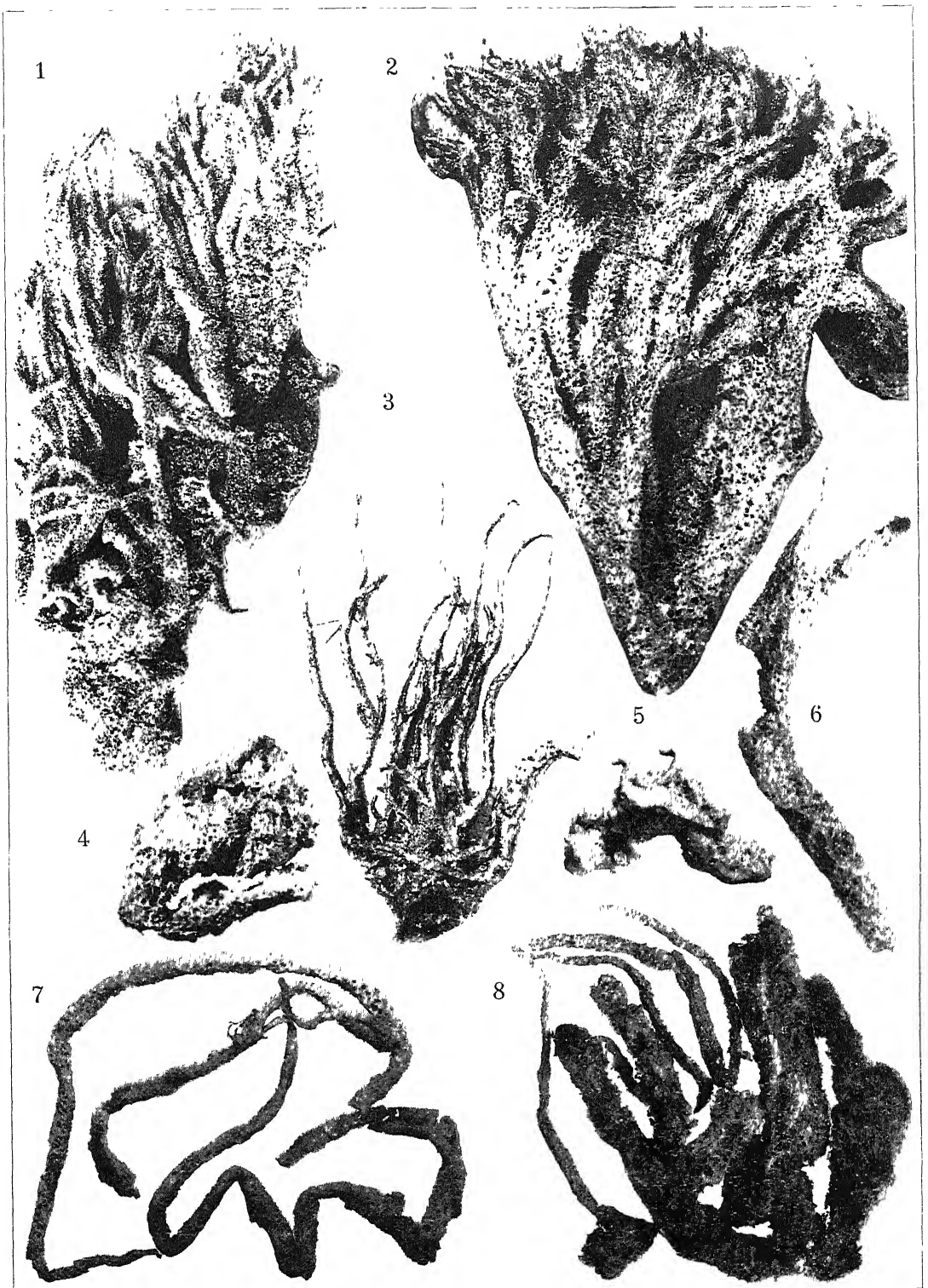
- Fig. 9. *Spongilla shikaribensis*, n. sp.; $\times 3/4$ Lake Shikaribetsu.
 Fig. 10. *Spongilla shikaribensis*, n. sp.; $\times 3/4$ Lake Shikaribetsu.
 Fig. 11. *Spongilla shikaribensis*, n. sp., $\times 3/4$ Lake Shikaribetsu.
 Fig. 12. *Spongilla fragilis* LEIDY and *Spongilla lacustris* (L.) $\times 1/4$ Lake Akan.
 Fig. 13. *Spongilla fragilis* LEIDY; $\times 3/4$ Lake Akan
 Fig. 14. *Spongilla fragilis* LEIDY, $\times 3/4$ Lake Akan
 Fig. 15. *Spongilla fragilis* LEIDY, $\times 3/4$. Small pond near Tomakomae.

PLATE VI.

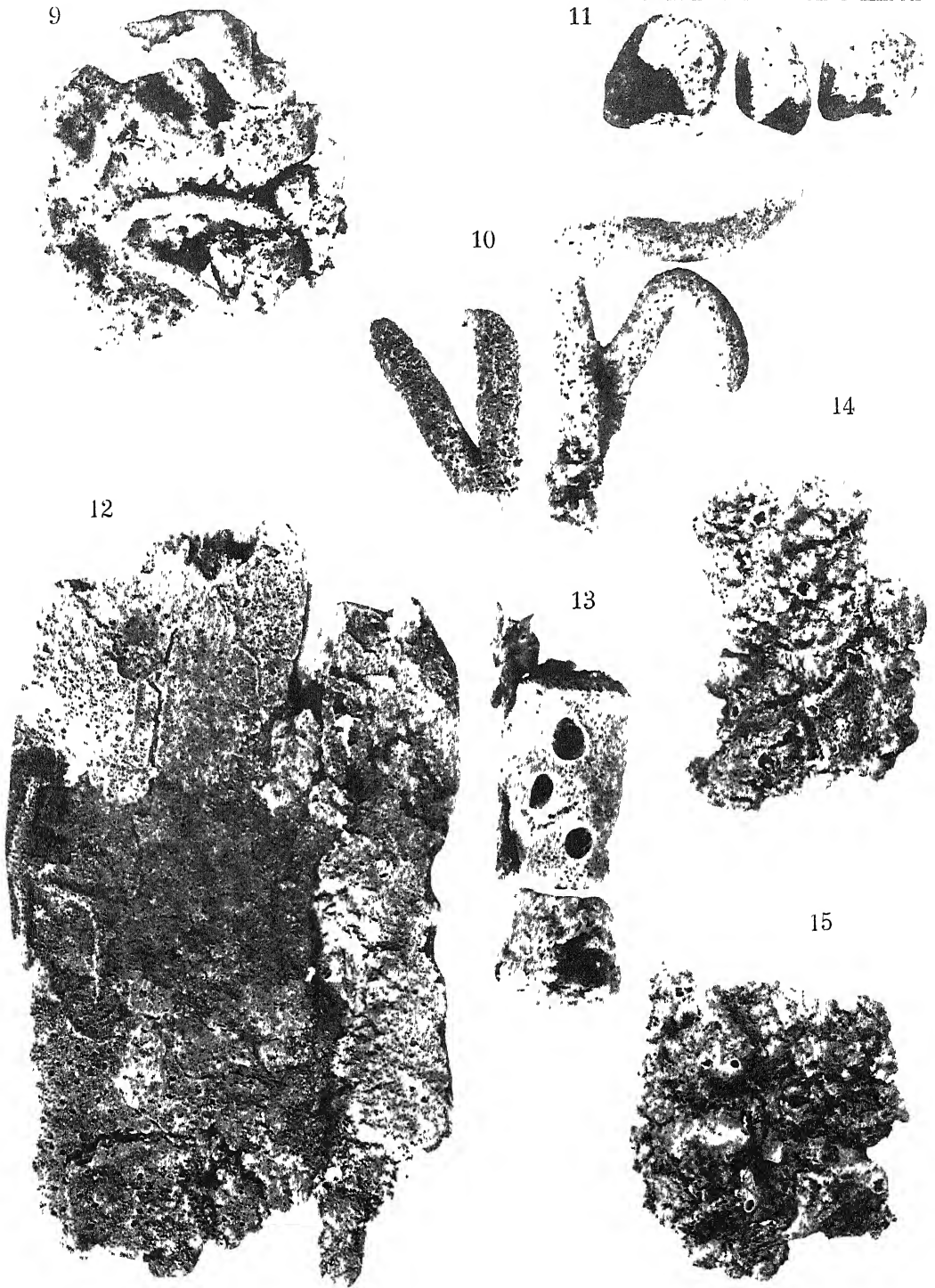
- Fig. 16. *Spongilla akanensis*, n. sp.; $\times 3/4$ Lake Akan.
 Fig. 17. *Spongilla akanensis*, n. sp.; $\times 3/4$ Lake Akan.
 Fig. 18. *Ephydatia fluviatilis* (L.), $\times 3/4$ Lake Abashiri.
 Fig. 19. *Ephydatia mulleri* (LIEBERKÜHN), $\times 3/4$ Lake Abashiri
 Fig. 20. *Ephydatia mulleri* (LIEBERKÜHN), $\times 3/4$ Lake Abashiri
 Fig. 21. *Ephydatia mulleri* (LIEBERKÜHN), $\times 3/4$ Lake Shikaribetsu.
 Fig. 22. *Ephydatia mulleri* (LIEBERKÜHN), $\times 3/4$ Lake Shikaribetsu
 Fig. 23. *Ephydatia mulleri* (LIEBERKÜHN), $\times 3/4$ Lake Shikaribetsu.
 Fig. 24. *Ephydatia mulleri* (LIEBERKÜHN), $\times 3/4$ Lake Shikaribetsu
 Fig. 25. *Ephydatia mulleri* var. *japonica* (HILGENDORF); $\times 3/4$ Lake Akan.
 Fig. 26. *Ephydatia mulleri* var. *japonica* (HILGENDORF), $\times 3/4$ Lake Abashiri
 Fig. 27. *Heteromeyenia baileyi* var. *petri* (LAUTERBORN); $\times 3/4$ Lake Abashiri.
 Fig. 28. *Heteromeyenia baileyi* var. *petri* (LAUTERBORN), $\times 3/4$ Lake Abashiri

PLATE VII.

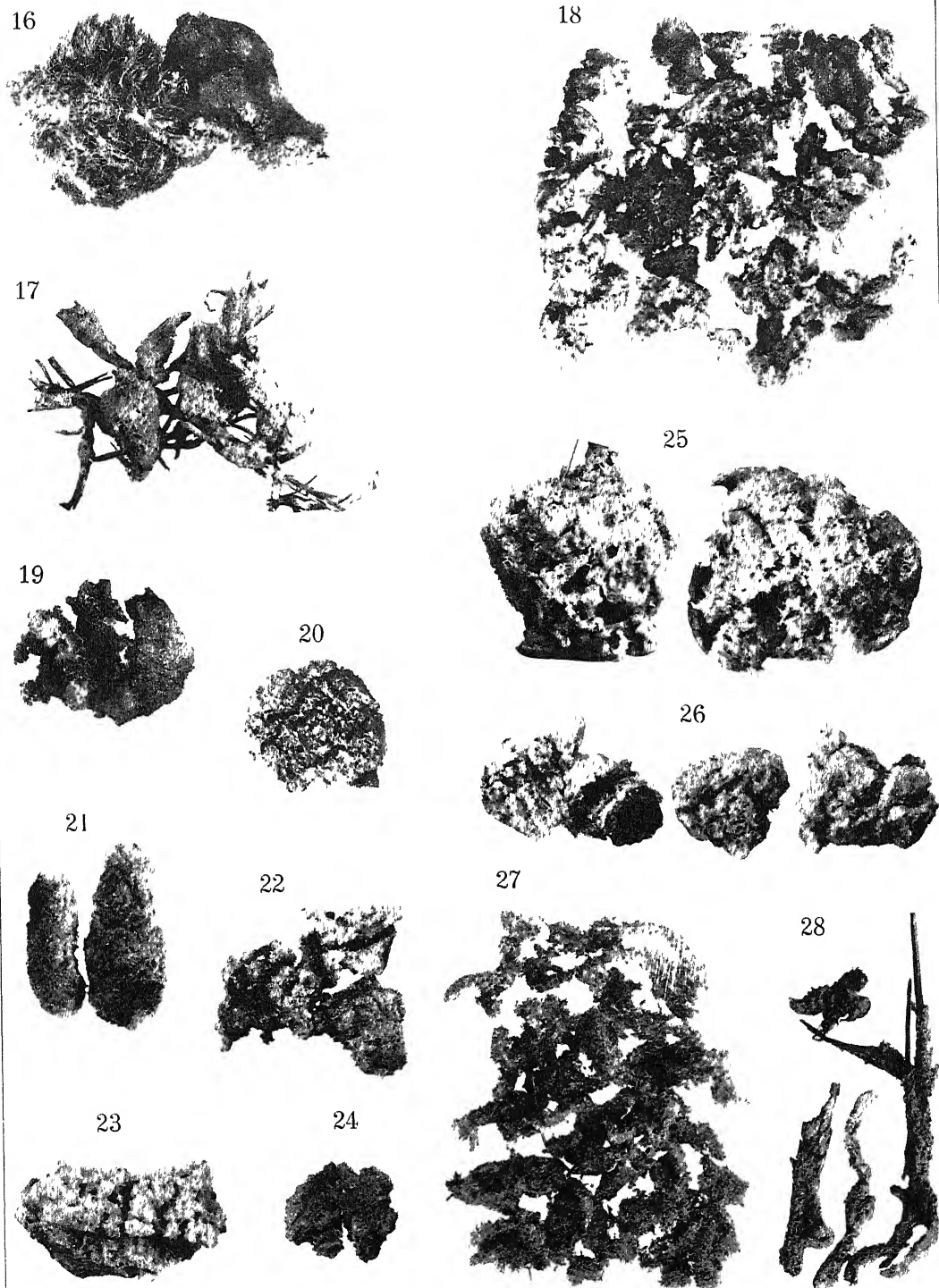
- Fig. 29. *Spongilla lacustris* (L.); Vertical section of foramen of the gemmule. $\times 225$. Lake Abashiri.
 Fig. 30. *Spongilla shikaribensis*, n. sp., Vertical section of foramen of the gemmule. $\times 225$. Lake Shikaribetsu.
 Fig. 31. *Spongilla fragilis* LEIDY; Vertical section of foramen of the gemmule. $\times 225$. Small pond near Tomakomae.
 Fig. 32. *Ephydatia fluviatilis* (L.), Vertical section of foramen of the gemmule. $\times 225$. Lake Abashiri.
 Fig. 33. *Ephydatia mulleri* (LIEBERKÜHN); Vertical section of foramen of the gemmule. $\times 225$. Lake Shikaribetsu.
 Fig. 34. *Ephydatia mulleri* var. *japonica* (HILGENDORF), Vertical section of the foramen of the gemmule. $\times 225$. Lake Akan.
 Fig. 35. *Heteromeyenia baileyi* var. *petri* (LAUTERBORN); Vertical section of the foramen of the gemmule $\times 225$. Lake Abashiri



Sasaki photo.

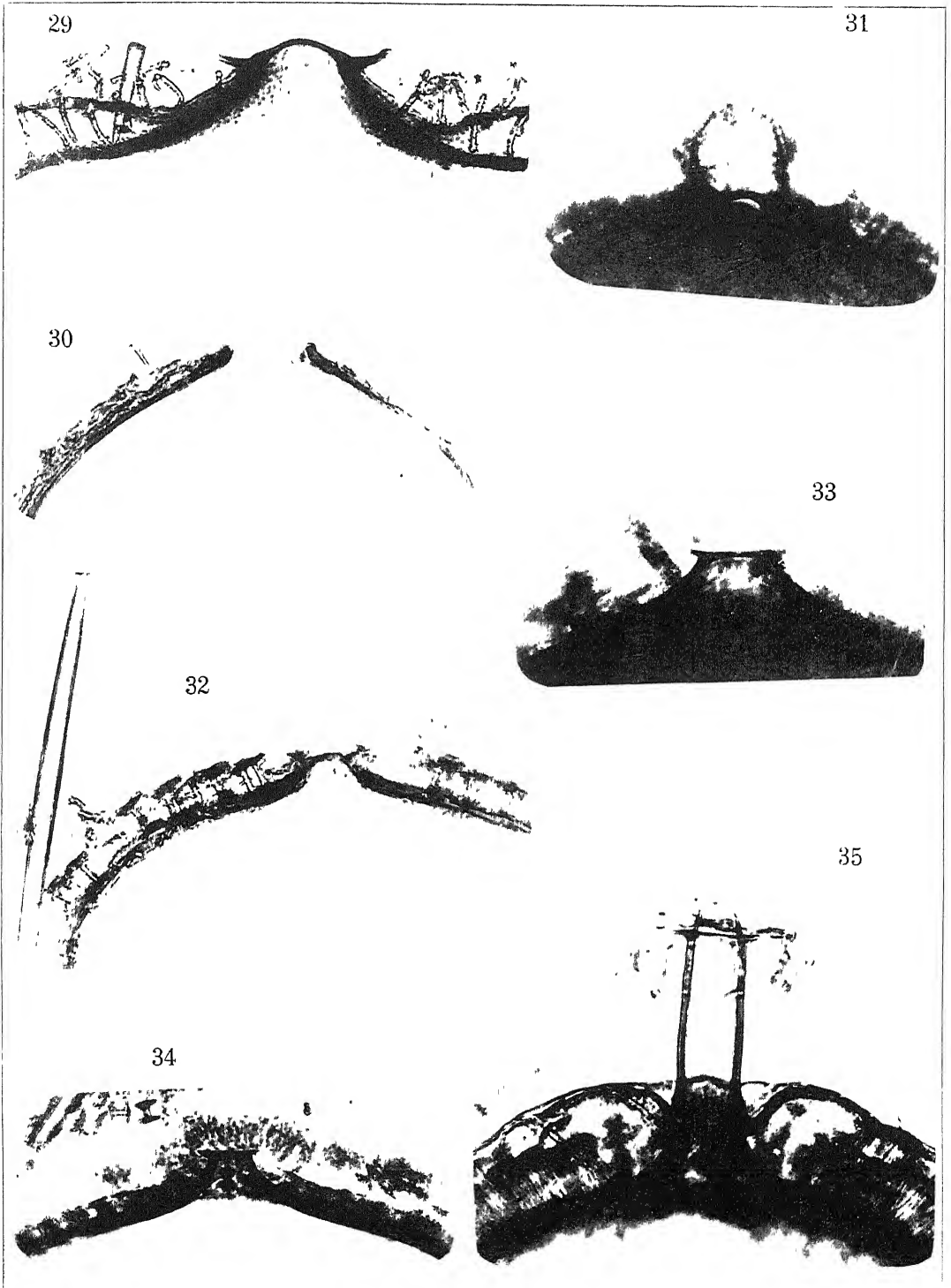


Sasaki photo



Sasaki photo.

N. SASAKI: Fresh-water Sponges of Hokkaido.



Sasaki photo.

N. SASAKI: Fresh-water Sponges of Hokkaido.

SUR LE POIDS DES SPORES CHEZ QUELQUES FOUGERES JAPONAISES

PAR

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(Reçu le 20 Août 1934)

Comme les plantes supérieures sont en général sédentaires, on comprendra facilement que, quand il s'agit pour elles de la reproduction de l'espèce, la dissémination en est aussi importante que la multiplication du nombre d'individus. Ce premier but est atteint ordinairement par les fruits ou les graines chez les phanérogames et par les spores chez les ptéridophytes. Les manières en sont extrêmement variées, dont les plus développées, se trouvant naturellement chez les phanérogames, sont toujours des objets d'admiration pour ceux qui s'intéressent à la vie des plantes. On remarquera, cependant, qu'il y a aussi des méthodes plus simples et plus primitives, dont l'extrême est celle de se disperser par la légèreté, c'est-à-dire, par le caractère des organes de dissémination qui peuvent se suspendre dans l'air grâce à la légèreté de leurs corps. Il y a des espèces de phanérogames chez lesquelles se trouve cette mode de dissémination. Mais, c'est dans la classe de fougères qu'on trouve cette méthode le plus généralement. Alors, c'est une question assez intéressante à étudier quel est le poids spécifique ainsi que le poids effectif des spores chez ces espèces de fougère. Comme il ne me semblait pas que cette question soit suffisamment étudiée chez nous, je me suis chargé d'en faire une petite recherche. J'ai ramassé des spores de quelques espèces ordinaires de fougère chez nous et en ai étudié les points susdits.

MATIERES ET METHODE

Les cinq espèces suivantes ont été employées pour mon étude : *Osmunda japonica* THUNB. (nom jap., Zen-mai), *Osmunda cinnamomea* L. (nom jap., Yamadori-zen-mai), *Woodwardia orientalis* SWARTZ (nom jap., Komoti-sida), *Dryopteris viridescens* O. KUNTZE (nom jap., Ryômen-sida) et *Equisetum arvense* L. (nom jap., Sugi-na). On a ramassé leurs spores dans les champs

et les purifiées d'après les indications données dans notre publication précédente¹⁾.

La méthode employée le plus souvent pour l'étude du poids spécifique des petits objets vivants est de préparer d'abord de la liquide de tel poids spécifique qui correspond à celui de l'objet qu'on va préciser, d'y faire suspendre le dernier, et ensuite de mettre cette suspension dans un centrifugeur. Quand les particules suspendues restent toujours uniformément distribuées dans la liquide, on peut prendre que le poids spécifique des premières est égal à celui de la liquide qui est déjà connu. BULLER, dans sa recherche sur les basidiomycètes²⁾, a déterminé le poids spécifique de leurs spores par cette méthode moyennant de la solution de quelques sels. J'ai essayé aussi cette méthode. Mais, ayant trouvé que, par cette méthode, on ne peut pas éviter la déformation des corps de spores dû à la plasmolyse et par conséquent la contraction de son volume, et en considération du calcul compliqué pour rectifier ce changement d'état, j'ai abandonné cette méthode et adopté une autre, c'est-à-dire, la méthode du flacon pour le poids spécifique. Je me suis servi, pour remplir le flacon, de la paraffine liquide qui n'interfère pas avec la spore. Quelquefois, quand on met dans le flacon d'abord des spores et ensuite cette liquide, on remarque qu'il y a de l'air qui reste dans l'amas des spores. En pareil cas, on met dans le vide tout le système, le flacon avec des spores et de la liquide dedans, et l'y laisse durant quelques minutes pour éliminer cet air embarrassant.

Les Spores de *Equisetum arvense*, de *Osmunda japonica* et de *Osmunda cinnamomea* sont sphériques, et il n'est pas difficile de déterminer leurs volumes. Celles de *Dryopteris viridescens* sont en forme de sphéroïde allongé, dont le calcul du volume est aussi assez facile à exécuter. Quant à *Woodwardia orientalis*, les spores sont assez ressemblantes à celles de *Dryopteris*, mais leurs formes ne sont pas très exactes, et leurs volumes ont été calculés approximativement comme si elles étaient les sphéroïdes réguliers. On a mesuré la longueur de l'axe de spore à l'aide d'une microscope de Zeiss (objectif DD et oculaire 5), muni d'un micromètre dans l'oculaire, et tous les chiffres qu'on va lire dans la deuxième colonne de la table ci-dessous sont des moyennes de dix mesurages.

¹⁾ OKADA, Y. Notes on the germination of the spores of some pteridophytes with special regard to their viability. Sci. Rep., Tôhoku Imp Univ., 4 ser., Vol. IV, pp. 127-182, 1927.

²⁾ BULLER, A. H. R.: Researches on Fungi, 1909, London. vol. I, p. 153.

RESULTAT

Le résultat est résumé dans la table suivante: —

Espèce	Longueur de l'axe en μ	Volume en mm^3	Poids spécifique	Poids effectif d'une spore en mg
<i>Equisetum arvense</i> L.	40	15×10^{-5}	1.26	19×10^{-5}
<i>Osmunda japonica</i> THUNB	50	29×10^{-5}	1.20	35×10^{-5}
<i>Osmunda cinnamomea</i> L.	47	24×10^{-5}	1.11	27×10^{-5}
<i>Woodwardia orientalis</i> SWARTZ	81.2×56.9	62×10^{-5}	1.09	68×10^{-5}
<i>Dryopteris viridescens</i> O. KUNTZE	49.8×35.1	14×10^{-5}	1.07	15×10^{-5}

En réalité, la forme de ces spores n'étant pas toujours régulière, les chiffres données ci-dessus doivent avoir quelques inexactitudes. Le fait qu'il y a eu, parmi les spores chez *l'Equisetum* de notre étude, quelques-unes qui gardaient les élatères, doit entraîner quelques erreurs aussi. Cependant, comme toutes ces erreurs ne sont pas, me semble-t-il, très graves, on peut considérer les chiffres ci-dessus comme quelque chose qui donne de la notion concernant le poids de spore chez les ptéridophytes.

ON A SNOW-PATCH ASSOCIATION AT MT. HAKKÔDA¹⁾

By

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(Pls. VIII-IX, four text-figures and five charts)

(Received September 20, 1934)

INTRODUCTION

Since a report by WAHLENBERG (1812)²⁾ on the snow-patch plant in the Alps was published, investigations along this line have been followed by a number of European workers, among these a recent work of ROMPEL (1928) is prominent. He describes in this paper mainly the efflorescence of many flowering and lower plants. Later the study of this problem in America was carried on by HARSHBERGER (1929), and he threw light upon this interesting and important field for ecologists, though the work was a preliminary account. Japan is one of the snow countries in the world, and affords a very convenient situation for the study of snow and plants, but there has been scarcely any study on this problem. This paper will give some results of observations on a snow-patch association at Mt. Hakkôda which were carried on in the summer of 1933.

On high mountains, generally, snow remains unmelted until late summer, and when it finally disappears, the ground is exposed suddenly to the strong summer heat till it is covered by snow again in autumn. Plants must grow up therefore during this short summer time, being covered by snow most of the remaining parts of the year. Under such conditions special associations are found in any high mountain region, and indeed the snow-patch association is one of the conspicuous plant communities there.

At Mt. Hakkôda snow falls early in October and disappears in June or July usually. There are two great snow-valleys at the north-east side of Ôdake, the highest peak of Mt. Hakkôda. One of these snow-valleys disappears at the end of August or early in September, while the other remains even till the middle of September. In a year of heavy snow fall, a part of this valley is covered with unmelted snow even in autumn and

¹⁾ Contributions from the Mt. Hakkôda Botanical Laboratory. No. 21.

²⁾ Cited in HARSHBERGER, p. 275.

meets the fresh snow of the coming season. In such a part of the valley no flowering plant comes out and only some kinds of moss and other lower plants are found. But in several places on the slopes of these valleys, snow-patch associations composed of many kinds of flowering plants are found. Investigation was carried out on a snow-patch association on a bank of one of these valleys from the 1st till the 16th of August in 1933. The inclination of this valley is about 26 degrees towards the direction of N 55° E. The place at which the observations were made is situated at the left bank of this valley, and inclines with an angle of 20 degrees towards the direction of N 80° E. The elevation is about 1450 meters.

1. METHODS

At the beginning of the observation, the snow-patch covered an area about 250 m. in length and about 30 m. in width. A stake was put at a point on the edge of this snow-patch as the starting point for the observations. From this point a 10 m. belt transect with a width of 1 m. was set outside of the snow-patch at right angles with the line of valley. The plants in this belt transect were observed carefully.

There are two methods for the study of plant successions in the field: One is to describe the changes of an association in a certain quadrat at intervals of time, and the other is to observe a series of quadrats one after another. While the former method should be adopted only when the succession goes rapidly or at least the greater parts of the succession process are observed, the latter is convenient for the observation of the different stages of the succession at the same time, although it has a fault in that this is an indirect method itself. In this investigation the plant succession was observed mainly by the former method in the nearest quadrat to the snow-patch. But as the observations must be done during a short time, the latter method was also adopted, i. e. the remaining quadrats of the belt transect were observed at the same time, and the results of these two methods were compared. In short, the first quadrat was charted and photographed carefully and accurately every few days, while the plants in the other quadrats of the transect were simply recorded at regular intervals during the investigation.

For the examination of shoots that came out under snow before melting, the snow at the border of the snow-patch was removed. Some young plants which sprouted in caves or gaps of snow were also examined there.

For the observation of the soil-temperature at the root layer and the

air-temperature near the ground, which are two important climatic factors for snow-patch plants, two simple meteorological screens were set in the field. One at the starting point, and the other at a point 5 m. away from this, at the center of the transect. Maximum-minimum thermometers were set in these two screens, and at the base of the screen soil temperatures were measured. Moreover observations for the air- and soil-temperatures at other places were also undertaken, though not every day. The water content of the soil at several places was also measured occasionally.

II HABITAT FACTORS IN THE MOUNTAINS

1. Weather

Mt. Hakkôda is one of the moist mountains in north Japan. Here prevail almost always the moistened winds from the Pacific Ocean and from the Japan Sea, and they often cause heavy fogs and rains. Sometimes even the north-wind is accompanied by cloud or rain, so that the blessing at fine weather is brought only by the south-wind. As the wind is very changeable here in summer, we rather seldom have fair weather from morning till night. The weather during the observation was as follows:

30th of July.	Cloudy	9th of August.	Cloudy
31st	Cloudy	10th	Fine and Cloudy
1st of August	Fine	11th	Fine and Cloudy
2nd	Cloudy	12th	Cloudy
3rd	Fine and Cloudy	13th	Cloudy and Rain
4th	Fine and Cloudy	14th	Cloudy
5th	Fine and Cloudy	15th	Rain and Cloudy
6th	Rain	16th	Cloudy
7th	Fine and Cloudy	17th	Rain
8th	Fine and Cloudy		

2. Snow-Patch

In 1933 the snow melted earlier than usual. On the first day of the observation, the 1st of August, snow covered an area about 250 m. in length and about 30 m. in width, but on the last day, the 16th of August, it had almost melted away. The results of the measurements showed that the distance between the starting-point and the border of the snow-patch increased every day about 86 cm. on the average, having shown it's maximum and minimum retreat for one day about 150 cm. and 50 cm. respectively. The width of the snow-patch, therefore, narrowed at the rate of about

twice this distance per day. Snow melts most actively in the evening, and indeed it was observed that the increase of the distance amounted to 20 cm. an hour in an evening. The data are given in Table I.

TABLE I.

	Distance between the starting point and the border of the snow-patch	Retreat of snow from the line of previous day	Rate of snow-melting per day
1/VIII	-0.14 m. ^a	—	—
2/VIII	0.40	0.54 m	0.54 m
4/VIII	3.35	2.95	1.48
6/VIII	4.32	0.97	0.49
9/VIII	7.60	3.28	1.09
12/VIII	9.75	2.15	0.72
16/VIII	12.80	3.05	0.76
1/VIII- 16/VIII		12.94 m.	0.862 m.

3. Air-Temperature

All the measurements of air-temperature were done at a height of 3-4 cm. above the ground. Maximum- and minimum temperatures are given in the screens at the starting point and at the center of the transect. The temperatures of these two points and of the border of the snow-patch were also measured at 11 o'clock in the morning. The results are given in Tables II and III. As will be seen from the table the temperature at the border of the snow-patch is generally about 2°C. lower and at the middle of the transect about 1°C. higher than at the starting point. It is worthy of note, therefore, that the air temperature of the border is always lower than at any other place in the field, although the difference is not large. The maximum- and minimum temperatures were nearly the same in both places, i. e. they fluctuated between 5.9°-16.4°C. As the measurements were not done every day, the daily range would be smaller than these values.

At the border of the snow-patch, we often met gaps between the snow and the ground. The air-temperature in these gaps was found to be proportional to their size. When the gap was nearly closed, the air-temperature was practically 0°C., but the temperatures denoted about 1°C., 3-4°C. and 7-8°C. in gaps of 1 cm. 2 cm. and 4 cm. in height respectively. In a snow-cave, a great gap under snow, the temperature was nearly the same or somewhat lower than that in the screen which stood far from the snow-patch.

TABLE II.

Air-temperatures at 11:00 A.M. (3-4 cm. above the ground).

	At the border of snow-patch	At the starting point	At the middle point, i. e. 5 m away from the starting point
1/VIII	17.5°C	—	25.2°C.
2/VIII	18.0	20.0°C	21.7
4/VIII	19.0	21.0	23.2
6/VIII	18.0	20.0	20.2
9/VIII	19.0	20.5	22.2
12/VIII	24.0	26.0	26.1
16/VIII	—	18.0	19.0

TABLE III.

Air-temperatures (3-4 cm. above the ground).

	At the starting point		At the middle point, i. e. 5 m. away from the starting point	
	Max	Min.	Max.	Min.
1/VIII- 2/VIII	20.0°C	11.1°C.	24.2°C.	13.9°C
2/VIII- 4/VIII	21.5	13.4	23.2	14.4
4/VIII- 6/VIII	26.0	14.1	26.7	14.6
6/VIII- 9/VIII	22.0	16.1	23.2	17.1
9/VIII-12/VIII	26.5	10.4	27.0	10.6
12/VIII-16/VIII	25.0	13.1	27.2	13.5
1/VIII-16/VIII	26.5	10.1	27.2	10.6

4. Soil-Temperature

Soil-temperatures were measured at depths of 1-1.5 cm. under ground. Results are summarized in Tables IV and V, and in Fig. 1. The soil-temperature near the border of the snow-patch is remarkable, but those far away from it are little worthy of note, as they are usually quite the same as the air-temperature or fluctuate only slightly from it.

Soil-temperature at the border of the snow-patch is about 5°C. usually, but as snow melts, it begins to rise rapidly. For example, on the 1st of August, soil-temperature at a point 10 cm. from the border of the snow-patch was as high as 15.5°C., while it showed only about 5°C. at the border.

snow-patch, and the soil-temperatures beyond this region remain always practically constant.

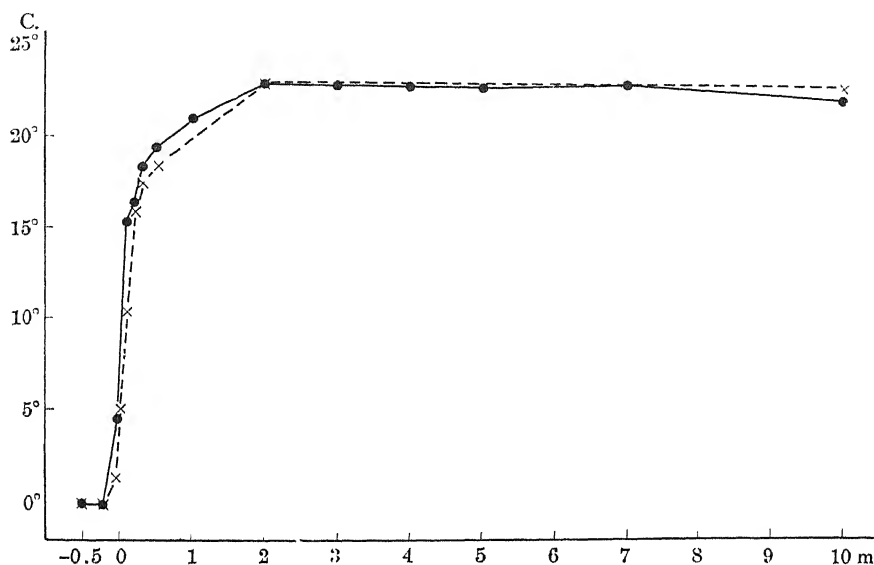


Fig. 1 Soil-temperature near the border of the snow-patch.

Ordinate — Soil-temperature.

Abscissa — Distance from the border of the snow-patch

—•— 1/VIII. - - - x - - - 9/VIII.

5. Temperatures of Snow and Thawing Water

The temperature at the surface of snow is very low in winter, but it is quite another thing in summer. The temperatures of snow in summer near the surface and in the interior are uniformly 0°C. As the snow in the snow-valley melts, the thawing waters run down under the snow-patch, and at last gather into a brook. The temperature of this thawing water is just 0°C. in the spring at the lower end of the snow-patch, but rises rapidly when the water is exposed to air. For example, the temperature of thawing water at a place 4 m. away from the spring had already risen to 1.5°C.

It is worth note, that the thawing water in a snow-cave of a melting bank is not very cold, showing about 4.5°C., and sometimes even more than 10°C.

6. Water Content of Soil

The water content of the soil was measured at several places, some

relation between water content and soil temperature being expected, especially at the border of the snow-patch, where the temperature is changeable. But the results proved quite otherwise, as far as the samples collected at a depth of 1-1.5 cm. under the surface show, for the soil was almost always saturated, and its water content was about 50-60 per cent, as given in Table VI.

TABLE VI.

Water content of soil in % (1-1.5 cm. under the ground).

	Under snow covering	Border of the snow-patch	Uncovered place							
			Distances from the border of the snow-patch in m							
			0.1	0.2	0.3	0.5	1.0	2.0	5.0	10.0
4/VIII	55.8	60.0	60.5	59.5	52.9	54.8	52.5	58.5	56.5	66.1
9/VIII	—	61.2	55.3	62.1	61.8	55.5	55.4	-	56.5	

III RESULTS OF THE INVESTIGATIONS

1. Aspect of the Vegetation in the Quadrat

a) On the 1st of August (Chart 1).

A part of the investigated place was covered by snow in the morning, but the whole was exposed in the afternoon. As soon as the snow disappeared a quadrat was laid out and charted. *Primula nipponica* and *Fauria Crista-galli* had sprouted already under snow, and their shoots were exposed to sun shine as the snow melted. The height of the shoots¹⁾ of *Primula nipponica* was about 6 mm. and the size of the plants was about 3 mm. The length of the shoots of *Fauria Crista-galli* was about 12 mm.

At the middle of this quadrat, 50 cm. from the starting point, where snow had retreated on the day before, the shoots of *Primula nipponica* had already begun to unfold, having reached a size at about 6 mm. and the length of the shoots of *Fauria Crista-galli* was about 6 mm.

At the end of this quadrat (1 m. from the starting point), where snow had retreated one or two days before, the shoots of *Fauria Crista-galli* had already reached 10-15 mm. in length, and the blade and stalk of leaves were clearly distinguishable. The leaf-blades began to unfold immediately. Almost the same was observed in *Primula nipponica*, but its center leaves

¹⁾In the following paragraphs the dimension of the plants is given on the average.

were not yet unfolded. No shoot of any other plants was found except a few small ones of *Carex blepharicarpa*. Old leaves of *Loiseleuria procumbens* remained withered there.

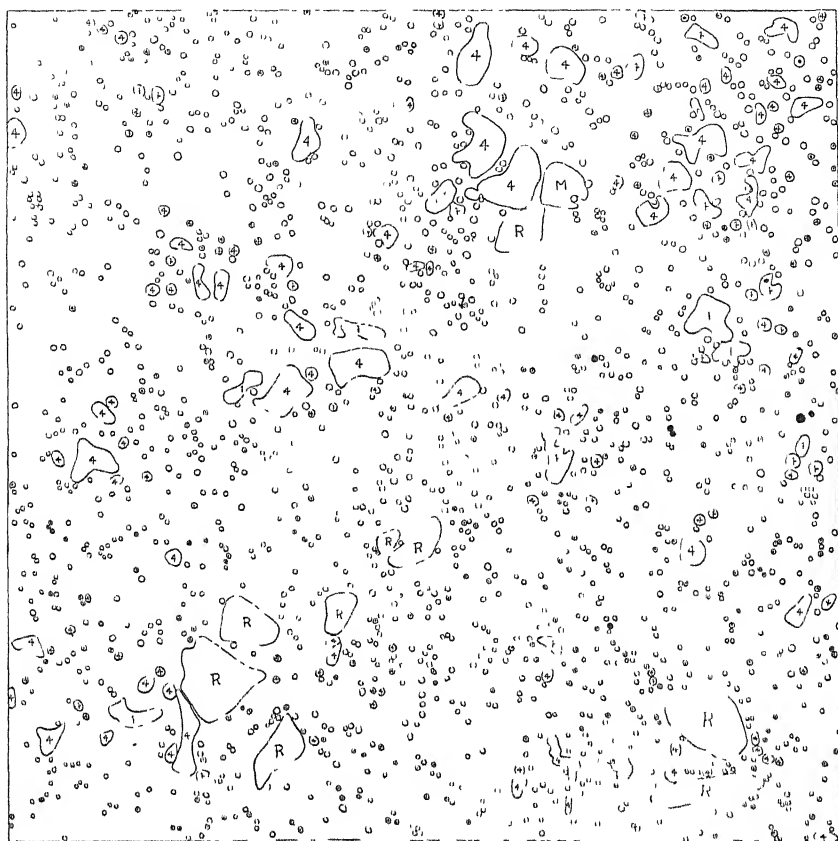


Chart 1. Quadrat on the 1st of August.

- | | |
|------------------------------|---------------------------------|
| () <i>Primula nipponica</i> | 4 <i>Loiseleuria procumbens</i> |
| ⊙ <i>Fauria Crista-galli</i> | M Moss. |
| ● <i>Carex blepharicarpa</i> | R Rock. |

The cover degree of each species in this quadrat was as follow: *Primula nipponica* (2), *Fauria Crista-galli* (1), *Loiseleuria procumbens* (2), *Carex blepharicarpa* (+).

b) On the 4th of August (Chart 2).

The snow at the starting-point had disappeared three days before, the distance between this point and the border of the snow-patch having reached already about 3.3 m. At the starting-point, the shoots of *Primula nipponica*

had quite opened, having reached about 30 mm. in size, and flower-buds were found. Leaf-blades of *Fauria Crista-galli* had half opened and the length of their stalks reached about 1 cm.

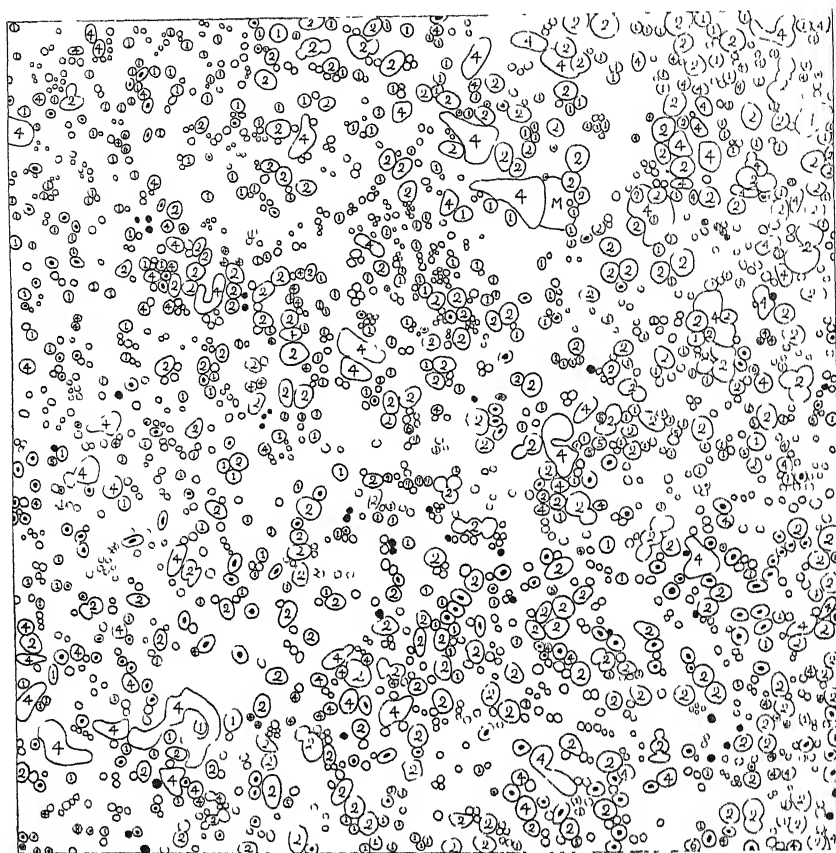


Chart 2. Quadrat on the 4th of August

- | | |
|-------------------------------------|-------------------------------------|
| 1 or ○ <i>Primula nipponica</i> | 6 <i>Pedicularis japonica</i> . |
| 2 or ⊙ <i>Fauria Crista-galli</i> , | 7 <i>Peucedanum multivittatum</i> . |
| 4 <i>Loiseleuria procumbens</i> . | 8 <i>Phyllodoce aleutica</i> . |
| 5 or ● <i>Carex blepharicarpa</i> . | |

At the center of the quadrat, flower-stalks of *Primula nipponica* appeared. Leaf-blades of *Fauria Crista-galli* were quite unfolded, and the length of leaves was about 5 cm. *Carex blepharicarpa* had grown up and reached about 30 cm. in height, but the shoots of *Pedicularis japonica* and *Peucedanum multivittatum* had only begun to unfold.

At the end of the quadrat, the flower stalks of *Primula nipponica* had

already reached about 10 mm. and the length of the leaves of *Fauria Crista-galli* showed about 7 cm. Even *Carex blepharicarpa* had reached 5 cm. in height, and the leaf-blades of *Pedicularis japonica* and *Peucedanum multivittatum* finally began to unfold.

The cover degree of each species was as follow: *Primula nipponica* (3), *Fauria Crista-galli* (3), *Carex blepharicarpa* (1), *Pedicularis japonica* (1), *Peucedanum multivittatum* (1), *Loiseleuria procumbens* (2).

c) On the 6th of August (Chart 3).

Five days had passed since snow disappeared from the starting-point,

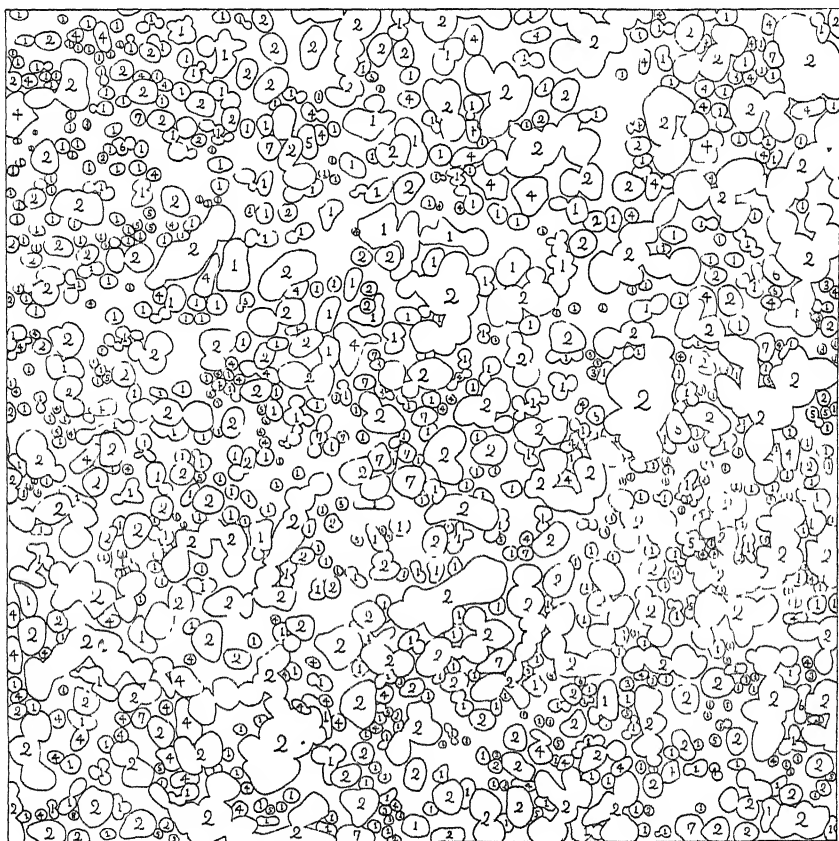


Chart 3. Quadrat on the 6th of August.

- | | |
|-----------------------------------|-------------------------------------|
| 1 <i>Primula nipponica</i> . | 5 <i>Carex blepharicarpa</i> . |
| 2 <i>Fauria Crista-galli</i> . | 6 <i>Pedicularis japonica</i> . |
| 3 <i>Geum pentapetalum</i> . | 7 <i>Peucedanum multivittatum</i> . |
| 4 <i>Loiseleuria procumbens</i> . | 8 <i>Phyllodoce aleutica</i> . |

and the distance between the starting point and the border of the snow-patch had reached about 4.3 m. The aspect of the vegetation at the starting-point was practically the same as that observed at the end on the 4th of August, except that shoots of *Geum pentapetalum* were found.

At the center of the quadrat, the flower-stalks of *Primula nipponica* had grown up about 20 mm., and leaves of *Fauria Crista-galli* were about 9 cm. and the height of *Pedicularis japonica* was about 2 cm., whereas *Peucedanum multivittatum* reached about 5 cm. Leaf-blades of *Geum pentapetalum* had not yet unfolded, but some flower-buds were found on *Carex blepharicarpa*.

At the end of the quadrat, the height of *Fauria Crista-galli*, *Pedicularis japonica* and *Peucedanum multivittatum* were about 10 cm., 3 cm., and 7 cm. respectively. The flower-stalks of *Primula japonica* reached over 35 mm. and its flower buds had grown large. *Geum pentapetalum* forth out flower-buds. *Carex blepharicarpa* was in flower.

The cover degree of each species was as follow: *Primula nipponica* (3), *Fauria Crista-galli* (4), *Geum pentapetalum* (1), *Carex blepharicarpa* (1), *Pedicularis japonica* (1), *Peucedanum multivittatum* (1), *Loiseleuria procumbens* (2).

d) On the 9th of August (Chart 4).

The distance between the starting-point and the border of the snow-patch advanced about 7.6 m. as the 8th day passed since snow disappeared at the starting-point. The leaves of *Fauria Crista-galli* completed their growth, and some flower-buds came out. While the flower-buds of *Primula nipponica* gradually began to open at the starting-point, some of them were in flower at the center, and the flowers were at their best at the end of the quadrat. The growth of leaves on *Geum pentapetalum* also nearly ended and many flower-buds appeared. *Pedicularis japonica* reached about 5 cm., and *Carex blepharicarpa* was in full flower.

The cover degree of each species was as follow: *Primula nipponica* (4), *Fauria Crista-galli* (5), *Geum pentapetalum* (1), *Carex blepharicarpa* (2), *Pedicularis japonica* (1), *Peucedanum multivittatum* (1), *Phyllodoce aleutica* (+), *Loiseleuria procumbens* (2).

e) On the 12th of August.

The distance between the starting-point and the border of the snow-patch reached as far as 9.75 m., 11 days having passed since the retreating

of the snow from the starting-point. As the growth of all the plants^{as in} the quadrat was nearly complete on this day, almost the same aspect was

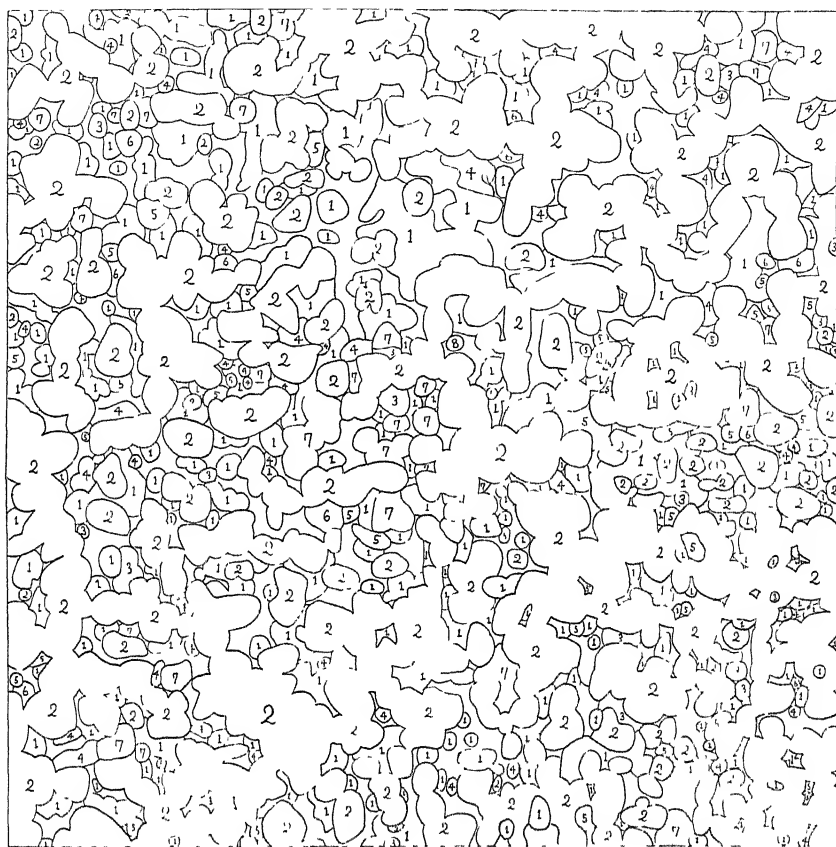


Chart 4. Quadrat on the 9th of August.

- | | |
|---------------------------------|-------------------------------------|
| 1 <i>Primula nipponica</i> . | 5 <i>Carex blepharicarpa</i> |
| 2 <i>Fauria Crista-galli</i> | 6 <i>Pedicularis japonica</i> . |
| 3 <i>Geum pentapetalum</i> . | 7 <i>Peucedanum multivittatum</i> . |
| 4 <i>Loiseleuria procumbens</i> | 8 <i>Phyllodoce aleutica</i> |

seen all over the quadrat. The flowers of *Primula nipponica* and *Carex blepharicarpa* were at their best.

The cover degree of each species was naturally the same as that on the 9th of August.

1) On the 16th of August (Chart 5).

The snow-patch had nearly disappeared and only a small mass of snow

was found at a point 12.8 m. away from the starting-point. Almost all the flowers of *Primula nipponica* were gone and only a few were found at the starting-point, and plenty of fruit was seen on *Carex blepharicarpa*. But *Fauria Crista-galli* was yet in flower, and finally the flower-buds of *Geum pentapetalum* had grown up, and even those of *Peucedanum multivittatum* came out. *Pedicularis japonica*, which grows most slowly in this

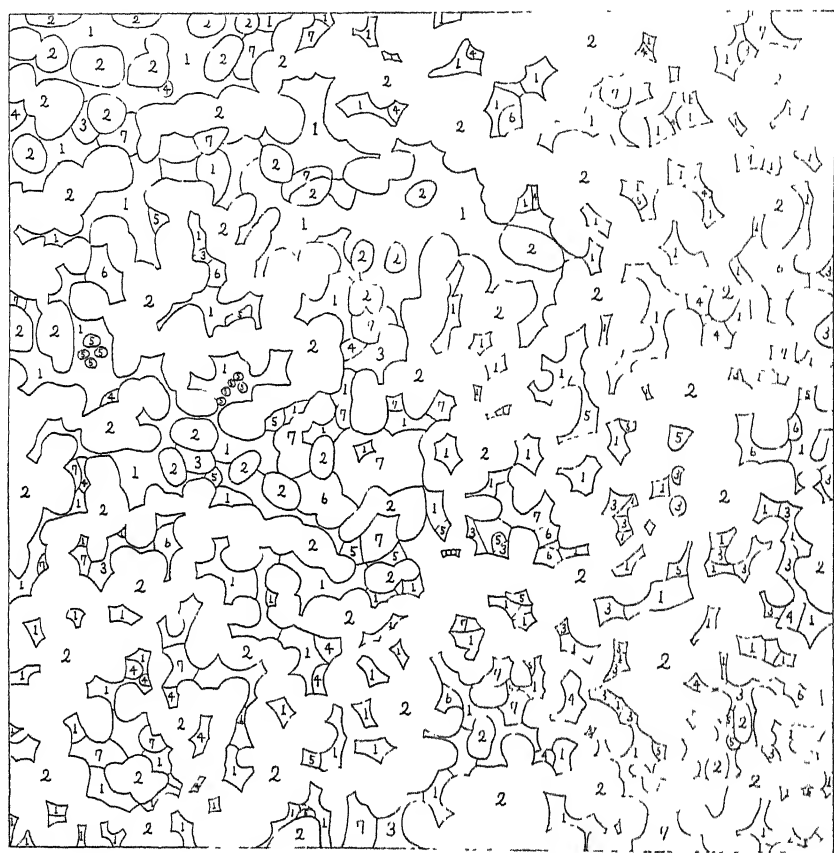


Chart 5. Quadrat on the 16th of August.

- | | |
|-----------------------------------|-------------------------------------|
| 1 <i>Primula nipponica</i> . | 5 <i>Carex blepharicarpa</i> . |
| 2 <i>Fauria Crista-galli</i> . | 6 <i>Pedicularis japonica</i> . |
| 3 <i>Geum pentapetalum</i> . | 7 <i>Peucedanum multivittatum</i> . |
| 4 <i>Loiseleuria procumbens</i> . | 8 <i>Phyllodoce aleutica</i> . |

association, reached 10–15 cm. Fresh shoots of *Loiseleuria procumbens* with many new buds were quite conspicuous at this time.

The cover degree of each species had not changed since the 9th of

August.

The quadrat was not observed any longer, for the development of this association was quite complete when the growth of *Pedicularis japonica* ended. In short, the plant succession of this association followed the following series. *Primula nipponica* and *Fauria Crista-galli* as the first exhibitors put forth their shoots under snow covering, and at the earliest stage the former played the greater part as the dominant species of the association, then the latter soon grew up and took the place of the former at the next stage. The leaves of *Fauria Crista-galli* when grown are usually eaten by insects, mainly by a kind of grasshopper. At the latter stage of the succession, *Geum pentapetalum* spread among the withered leaves of *Fauria Crista-galli*. At that time *Primula nipponica* was almost withered, and no fresh leaf was found there (Table VII. fig. 2).

Therefore, three stages are recognized in the succession of this snow-patch association: The first of *Primula nipponica*, the second of *Fauria Crista-galli*, and the last of *Geum pentapetalum*.

TABLE VII.

Aspect of the vegetation in the quadrat.
Numbers in parenthesis show the cover degree.

	1/VIII	4/VIII	6/VIII	9/VIII	12/VIII	16/VIII
<i>Primula nipponica</i>	Shoots began to unfold (2)	Flower-buds (2)	Flower-buds began to unfold (3)	Flowers (4)	In full flower (4)	Flowers were gone (4)
<i>Fauria Crista-galli</i>	Shoots (1)	Leaf-blades began to open (3)	(4)	Flower-buds (5)	(5)	Flowers (5)
<i>Geum pentapetalum</i>			Shoot (1)	Leaves opened, Flower-buds (1)	(1)	(1)
<i>Carex blepharicarpa</i>	Shoots (+)	(1)	5 cm. in height Flower-buds (1)	7 cm in height Flowers (2)	(2)	Fruits (2)
<i>Pedicularis japonica</i>		Shoots (1)	Leaves unfolded (1)	5 cm. in height (1)	(1)	10-15 cm. in height (1)
<i>Peucedanum multivittatum</i>		Shoots (1)	Leaves unfolded (1)	(1)	(1)	Flower-buds (2)
<i>Phyllodoce aleutica</i>				Shoots (+)	(+)	(+)
<i>Loiseleuria procumbens</i>	(2)	(2)	(2)	New leaves (2)	(2)	(2)

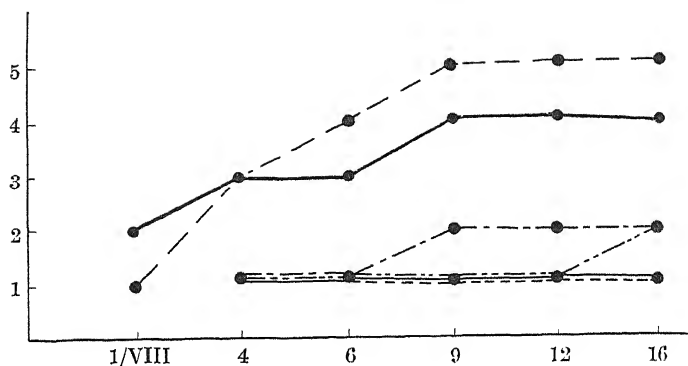


Fig. 2. The Cover degree of the main species in the quadrat

Ordinate	Cover degree.	-----	<i>Geum pentapetalum</i> .
Abscissa	Date of the observation	<i>Carex blepharicarpa</i>
————	<i>Primula nipponica</i>	<i>Pedicularis japonica</i>
-----	<i>Fauria Crista-galli</i>	<i>Peucedanum multivittatum</i>

2. Aspects of the Vegetation in the Belt Transect (Table VIII).

a) On the 1st of August.

The end quadrat of this 10 m. belt transect was exposed as a result of snow melting about two weeks before, i.e. in the middle of July. The aspect of the vegetation in the first 1 m. quadrat was described in the previous chapter, so it needs no further description.

Aspects of the vegetation between 1 m. and 15 m. away from the starting-point were nearly the same as in the first quadrat, and indeed there were to be found no plants, except *Primula nipponica* and *Fauria Crista-galli*. *Geum pentapetalum*, *Peucedanum multivittatum* and *Carex blepharicarpa* were found only at 2 m. away from the starting point, and at 2.5 m. *Primula nipponica* had already many fairly large flower-buds, and *Fauria Crista-galli* had grown about 1 cm. high. At 3 m. *Fauria Crista-galli* opened its leaves completely, and *Primula nipponica* and *Carex blepharicarpa* began to flower, and moreover *Phyllodoce aleutica* had flower-buds. Almost the same aspect was shown till about 5 m. away from the starting-point, and indeed here the flowers of *Primula nipponica* and *Carex blepharicarpa* were at their best, and *Geum pentapetalum* and *Peucedanum multivittatum* had grown up. At 7 m. the flowers of *Primula nipponica* began to fall, and at 8 m. those of *Primula nipponica* as well as *Carex blepharicarpa* were gone completely. At 9 m. *Geum pentapetalum* and *Tofieldia Okuboii* were in flower, and *Fauria Crista-galli* had large

flower-buds. At the end of this belt transect, 10 m. away from the starting-point, flowers of *Geum pentapetalum* were over, but those of *Phyllodoce aleutica* and *Shortia soldanelloides* var. *genuina* f. *typica* were still out.

b) On the 9th of August.

The distance between the starting-point and the border of the snow-patch was about 7.6 m. Therefore, the aspect of the vegetation at the starting-point on this day would be assumed to be the same as that at 7.6 m. from the starting-point on the 1st of August, but in reality it was nearly the same as that at 5 m. on that day. This is owing to the fact that the speed of snow-melting in July is slower than in August.

As above stated on the 1st of August, in the end of the transect the flowers of *Primula nipponica* and *Carex blepharicarpa* were over and plenty of fruit was seen. Almost the same aspect as this was shown at the center of this transect, i. e. at 5 m. from the starting-point. But here, *Tofieldia Okuboii* was in flower, and the growth of *Peucedanum multivittatum* and *Pedicularis japonica* was almost completed. At 7 m. *Peucedanum multivittatum* and *Phyllodoce aleutica* were in flower but at 9 m. *Geum pentapetalum* had fruit, and at the end of the transect even *Shortia soldanelloides* v. *genuina* f. *typica* fructified.

As the aspects of the vegetation on both sides of the snow-patch are practically uniform in nature, so we will find zones surrounding the snow-patch, if we look at it from a distance (fig. 3). The inner most zone of earth-colour is 1.5–2.0 m. wide, and has only some small shoots of *Primula nipponica* and *Fauria*

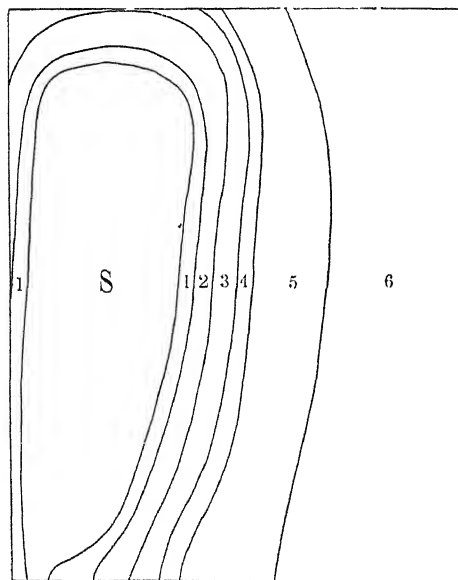


Fig 3. Schema of the plant zones surrounding the snow-patch.

- S. Snow-patch.
- 1 Zone of earth-colour.
- 2 Green zone.
- 3. Distinctly white zone.
- 4. Green narrow zone.
- 5. Dull white zone.
- 6. Outer field.

Crista-galli surrounding the snow-patch directly. Outside of this lies a green zone about 1.5 m. wide, which is composed of the grown up shoots of those two plants mixed with other herbs. This is surrounded by a distinct white zone of about 2.5–3.0 m. width, which originates from the pretty flowers of *Primula nipponica* at their best. Outside of this again lies a narrow green zone, which is composed of the withered flowers of *Primula nipponica*. A dull white forms the outermost zone, and here *Geum pentapetalum* is in flower. But the boundary of this zone is not so clear as the former ones, continuing into the green vegetation of the field.

3. The Snow-Patch Plants under Snow.

Some plants can grow easily under a snow covering if there is a gap between the snow and the ground, as described before. Now the results of the observations on the shoots of *Primula nipponica* and *Fauria Crista-galli* under snow will be described. For facility of observation, the snow near the border of the snow-patch was first removed. The depth of snow covering here at 1 m. inside the border, was about 30 cm.

As mentioned above, at the border of the snow-patch the height of the shoots of *Primula nipponica* was about 6 mm, their size was about 3 mm. and the length of a shoot of *Fauria Crista-galli* was about 3 mm. No shoot of *Fauria Crista-galli* was found inside of 30 cm. but many shoots of *Primula nipponica* were found even at 1 m. inside the border, and they had reached already a height of about 4–5 mm. and a size of about 2 mm. The development of the shoots under snow at 50 cm. as well as at 30 cm. inside was practically the same as that at the border of the snow-patch. From this, it is found that *Primula nipponica* can grow at more than 1 m. inside a snow-patch where the depth of snow-covering reaches over 30 cm.

IV LIFE-CYCLE OF THE SNOW-PATCH PLANTS.

The rate of growth of plants near the snow-patch is very rapid; some plants grow up within only three weeks, and even the plants which grow most slowly need only one and a half months for fructification after sprouting. The snow-patches in this place generally disappear at the end of August or early in September, and snow falls early in October. Therefore the plants are able to grow only one and a half or two months here, and they may be called true snow-patch plants (HARSHBERGER p. 276).

The growing periods of these snow-patch plants will be described in the following paragraphs.

a) *Primula nipponica*.

Shoots were put forth under snow more than two days before the snow melted, and began to unfold within a half day after being exposed to air. Some flower-buds were found on the 4th day, and flower-stalks grew up on the next day, flowers came out on the 9th day and began to fall on the 15th day and were all gone on the 16th day.

b) *Fauria Crista-galli*.

Shoots of this also were put forth under snow before melting. Leaves began to unfold on the 3rd day, and opened completely on the 5th day. Flower-buds were found on the 10th day. Flowers were at their best on the 15th day and fall mostly on the 20th day.

c) *Carex blepharicarpa*.

Shoots were put forth on the 4th day after the snow melted, but sometimes were found already on the 2nd day, and then grew very fast. Many flower-buds were seen on the 3rd day, flowers appeared on the 4th day and plenty of fruit was found on the 11th day.

d) *Geum pentapetalum*.

Shoots appeared on the 5th day after the snow melted. Leaves unfolded on the 9th day, and some flower-buds were found on the 11th day, flowers were opened on the 15th day, but had gone by the 18th day, and much fruit ripened on the 22nd or on the 23rd day.

e) *Peucedanum multivittatum*.

Shoots were put forth on the 5th day after the snow melted. Leaves unfolded completely on the 10th day, many flower-buds came out on the 17th day, and were all gone already on the 20th day.

f) *Pedicularis japonica*.

The shoots came out on the 5th day after the snow melted. Leaves finally unfolded completely on the 20th day. This is the plant which grows most slowly in this association.

g) *Tofieldia Okuboi*.

The day of sprouting was not ascertained, for there were very few plants. Flowers were gone on the 15th day after snow-melting.

h) *Shortia soldanelloides* v. *genuina* f. *typica*.

The day of sprouting for this also was not ascertained, for the plant was rare in the association. Flowers fell on the 15th day and fruit came out on the 22nd or 23rd day after the snow melted.

i) *Pholodoce aleutica*.

This is an ever-green plant. Flower-buds were found on the 4th day and flowers were gone on the 15th or 16th day after snow-melting.

j) *Loiseleuria procumbens*.

This is another ever-green plant. A fairly great number of these plants was found but they were all almost dying, and no flower was found.

V. SOIL-TEMPERATURE, LIGHT-INTENSITY AND THE GROWTH OF PLANTS

Although the air-temperature and water content of soil must play a great part in the growth of perennials before and after sprouting, the results of the observations showed on the one hand that the difference of air-temperature between the border of the snow-patch and places far away from it was little, and on the other hand that the water content of the soil was nearly the same at any place whether it was tested under snow or at exposed places, for the soil was always saturated. Therefore neither the air temperature nor the water content of the soil controls the change of the aspect of the vegetation, at least in this case.

1. Soil-Temperature and Plants.

The soil-temperature changes suddenly near the border of the snow-patch (Table IV and fig. 1). The relation between this sudden change of soil-temperature and the aspects of vegetation will be discussed in the following paragraphs. The growth rates of *Primula nipponica*, *Fauria Crista-galli* and *Carex blepharicarpa* are given in Table IX and fig. 4.

TABLE IX.

Sizes of some plants at various spots (in mm.).

		Under snow covering, distances from the border of the snow- patch (in m)			Border of the snow- patch	Uncovered place, distances from the border of the snow-patch (in m)											
		0.5	0.3	0.1		0.1	0.15	0.2	0.3	0.5	1.0	2.0	3.0	5.0	7.5		
Primula nipponica	Height	4-5	5-6	5-6	4-6	4.6	4.8	8	8	10				20			
	Diameter	2	2-3	2-3	3	3	3-4	4	4	6	8-10			25			
Fauria Cri- sta-galli	Height		0.5	—	1-2	3	—	—	5	6	10-15	30	70	90	150		
Carex ble- pharicarpa	Height									3-10			30	50	60		

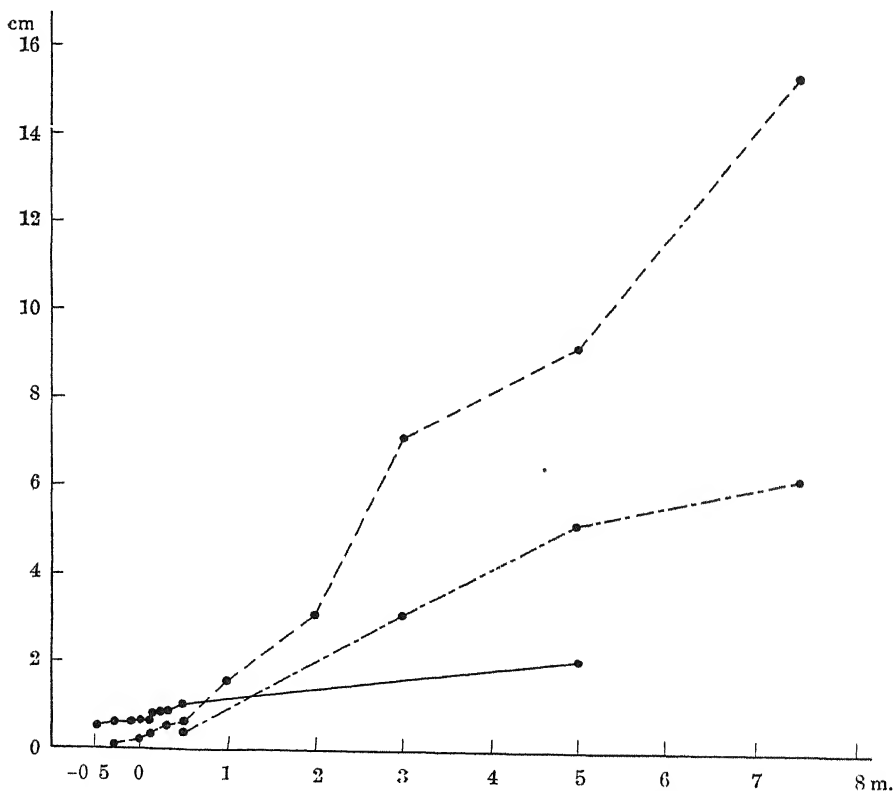


Fig 4. The relation between the sudden change of soil-temperature near the border of the snow-patch and the growth rate of some plants.

Ordinate Size of the plant

Abscissa Distance from the border
of the snow-patch

----- Primula nipponica.

- · - · - Fauria Crista-galli.

———— Carex blepharicarpa

a) *Primula nipponica*.

At a point 1 m. inside the border of the snow-patch the height and size of this plant showed about 4–5 mm and 2 mm. respectively. Now the snow at this place would disappear within 2 days, so we can assume that this plant had sprouted out under a deeper snow covering at a recent day. At 30 cm. inside the border the height and size of the shoots was about 5–6 mm. and 2–3 mm. respectively. At the border, however, they reached about 6 mm. and 3 mm. respectively. From these data, we are convinced that the shoots grow very slowly under snow at a temperature of just 0°C.

While the shoots at the border and at 10 cm. away from it were almost the same height, those at 15 cm. reached about 8 mm. in height and 4 mm. in size. But the shoots 50 cm. away from the border had a height of about 10 mm. and a size of about 6 mm. From this it is proved that the growth of this plant after the snow melts is remarkably rapid, presumably beginning within an hour after the snow is gone. While the soil-temperatures at 10 cm. and at 5 cm. inside of the snow-patch were 0°C. and 5°C. respectively, it registered 15.5°C. at 10 cm. away from the border. From these facts, we are convinced that the growth of the plant is affected principally by the rise of soil-temperature, and measurable change in the size of a shoot takes place within one or two hours when the soil-temperature rises.

b) *Fauria Crista-galli*.

At 30 cm. inside the snow-patch, where the soil-temperature was just 0°C., many shoots were put forth under snow. Two hours afterwards, snow having disappeared, the shoots of 1–2 mm. were exposed to the open air. At the same time shoots of about 3 mm. were seen at 15–20 cm. from the border. The growth of the shoots was remarkably rapid within about three hours after the rise in soil-temperature. Yet the reaction of this plant to the rise in soil-temperature was not so distinct as *Primula nipponica*.

c) *Carex blepharicarpa* and others.

The shoots of *Carex blepharicarpa* were first found at a place from which snow had disappeared only one day before. The soil-temperature was about 19°C. This plant did not react to the rise in soil-temperature so quickly as the former plants. The other plants in the snow-patch sprout out gradually in three to five days after the snow melts. Thus the reaction of these plants to the rise of soil-temperature is not noticeable, yet it is quick enough compared with ordinary plants.

2. Light Intensity and Sprouting.

The most important factor in the growth of shoots of *Primula nipponica*, *Fauria Crista-galli*, and others is the soil-temperature. But the sprouting of *Primula nipponica* and *Fauria Crista-galli* is not primarily caused by the rise of soil-temperature. As mentioned above, they shoot out under snow of just 0°C., the temperature which has remained practically constant under the snow covering since the past winter. Therefore, probably light plays a great part in the growing of these plants under snow. As snow melting proceeds in summer the ground under snow becomes lightened and this facilitates the sprouting of the shoots which have been at rest in the winter period.

The relative light intensity under snow has been studied by RUBEL, and later was measured more precisely by GOTZ who came to nearly the same result.

Now at the place where *Fauria Crista-galli* sprouted the thickness of the snow covering was about 10 cm. so that the relative light intensity was assumed to be about 1/30, whereas *Primula nipponica* appeared under a snow covering of more than 40 cm. thickness so that, the relative light intensity must have been less than 1/300, according to the data of RÜBEL. These young shoots under snow are always green with chlorophyll; probably they can assimilate at any rate under snow. Many interesting problems concerning such shoots under snow remain for further investigation.

VI PLANTS IN SNOW-CAVES AND THAWING WATER.

There were few caves or large gaps under snow on the lower banks of the snow-patch. The results of the observations undertaken in these snow-caves and in thawing water will be described here.

Primula nipponica and *Lysichiton camtschatense* are sometimes in flower in these snow-caves or gaps. As mentioned above, the air therein is not so cold as one would expect. Air-temperature in the caves where *Primula nipponica* or *Lysichiton camtschatense* were in flower was usually 7–10°C., whereas it was about 15°C. at any place outside the snow-patch. *Lysichiton camtschatense* was found usually in thawing water in a cave of about 2–5°C. which rose sometimes to 10°C. It is noteworthy that the plant did not flower at any place cooler than 2–5°C. The leaves of *Lysichiton camtschatense* in a snow-cave are mostly yellowish white, but those of *Primula nipponica* are usually green.

Thawing water which sprang from the lower part of the snow-patch

ran down as a brook, and the water-temperature was just 0°C. Even in this cold water, spore formation of a moss was observed.

I wish to express my hearty thanks to Prof. Y. YOSHII, under whose direction this work was made.

SUMMARY

1) A snow-patch association was investigated in a snow valley on the north-east side of Otake, the highest peak of Mt Hakkôda. The elevation of this place is about 1450 m. Here snow falls early in October and disappears in the end of August or early in September every year.

2) The investigation was undertaken from the 1st to the 16th of August in 1933. At the beginning the area of the snow-patch was about 250 m. in length and about 30 m. in width, but all the snow disappeared during the investigation. A 10 m. belt transect 1 m. wide was set from the border of the snow-patch at right angles with the line of the valley, and the plant succession was observed.

3) The weather in this mountain region was very changeable. Air-temperature at 3–4 cm. above the ground at the border of the snow-patch was nearly the same as that far away from it, and was about 18–19°C. on the average. Soil-temperature at a depth of 1–1.5 cm. at a place far from the snow-patch was nearly the same as air-temperature, while it was just 0°C. at 20 cm. inside the snow-patch. Soil-temperature changed suddenly near the border of the snow-patch, especially between 5 cm. inside and 10 cm. outside.

4) The growth rate of the plants is very rapid. Most of them end their life within only three weeks, and even the plants which grow most slowly need only one and a half months. 10 species were found in this association, two belonging to ever-green plants. Almost all plants put forth shoots on the 4th or 5th day after the snow melts. *Primula nipponica* and *Fauria Crista-galli* had already sprouted under snow covering, and grew rapidly after the snow melted. This was caused mainly by the sudden rise of soil-temperature.

5) The sprouting of *Primula nipponica* and *Fauria Crista-galli* are effected by the increase of light intensity under snow. *Primula nipponica* is able to sprout even under a snow covering of more than 40 cm. where the relative light intensity is assumed to be about 1/150–1/400. At that time it is green with chlorophyll.

6) *Primula nipponica* and *Lysichiton camtschatense* are sometimes in flower in caves under snow. The air-temperature in such caves is compara-

tively high, and indeed is usually 7–10°C. *Lysichiton camtschatense* is found in the thawing water in snow-caves where the water-temperature is 2–10°C. Spore formation of a moss is observed even in thawing water of 0°C on the lower bank of the snow-patch.

LITERATURE

- HARSHBERGER, J. W., Preliminary notes on American snow patches and their plants Ecology Vol X, p 275, 1929.
 ROMPEL, J., Beobachtungen über die bis zum Aufblühen alpiner Arten verstreichende Aperi-zeit Österr. Bot. Zeits Bd LXXVII, p. 178, 1928.
 RÜBEL, E., Lichtklima und Lichtgenuss. Hand. d. biol. Arbeitsmethoden, Abt. XI, Teil 5., p. 233, 1928.

EXPLANATION OF THE PLATES

PLATE VIII

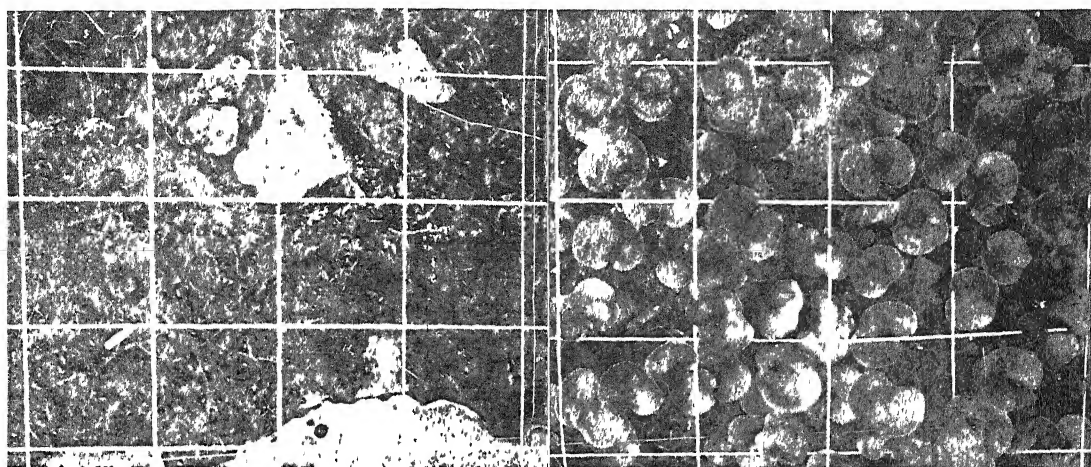
Aspect of the vegetation at the same place (0–0.3 m away from the border) in the quadrat at intervals of time.

1. On the 1st of August
2. On the 4th of August
3. On the 6th of August
4. On the 9th of August
5. On the 12th of August.
6. On the 16th of August.

PLATE IX.

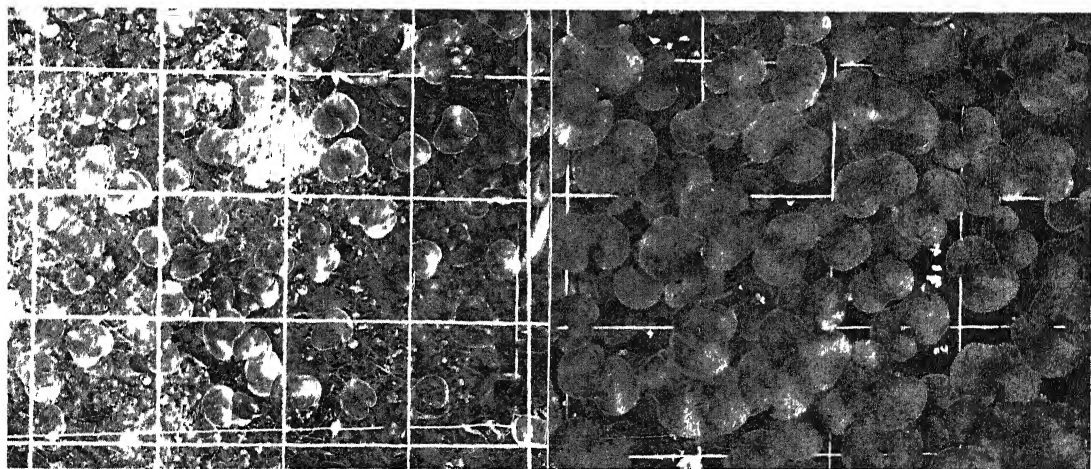
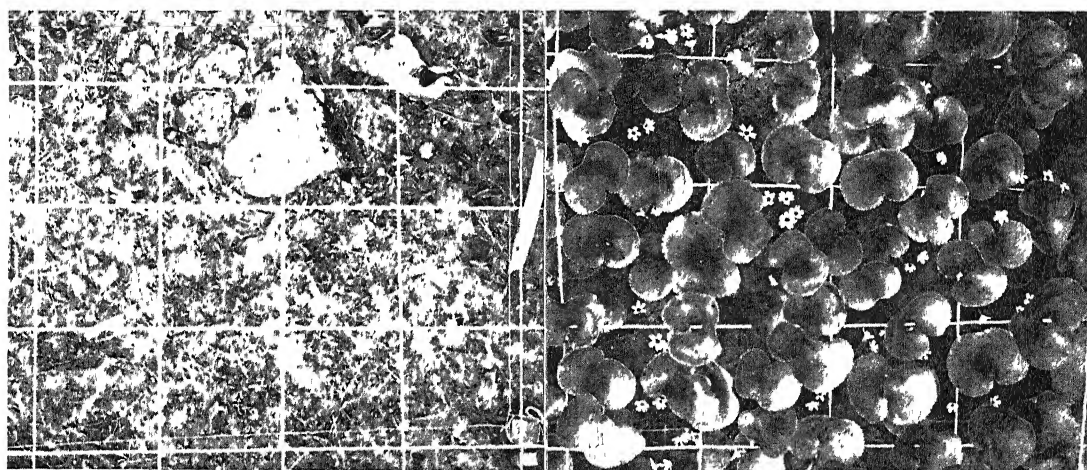
Aspect of the vegetation at various parts in the belt transect at the same time.

- | | | |
|----|---|---|
| 7 | 1–1.3 m away from the border of the snow-patch. | |
| 8 | 2–2.3 m | " |
| 9 | 5–5.3 m. | " |
| 10 | 7–7.3 m. | " |
| 11 | 9.7–10 m | " |
| 12 | About 30 m. | " |



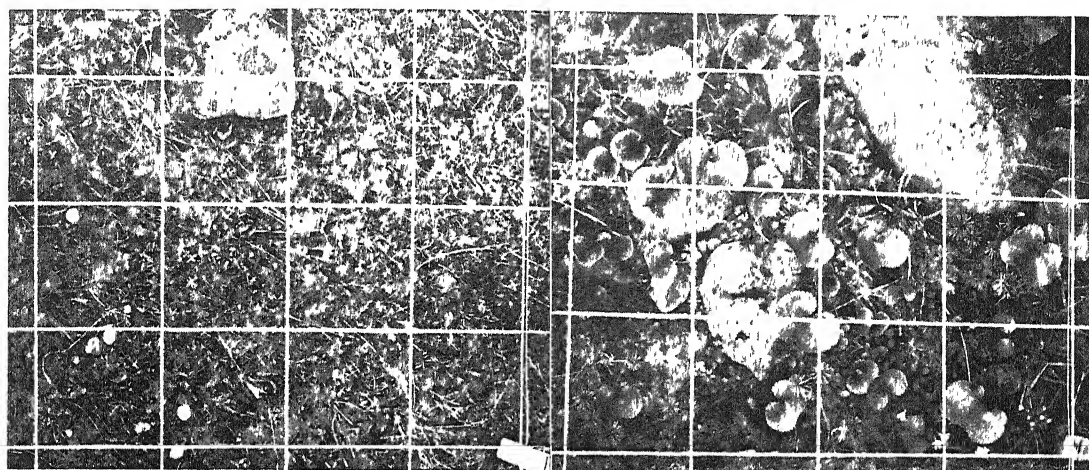
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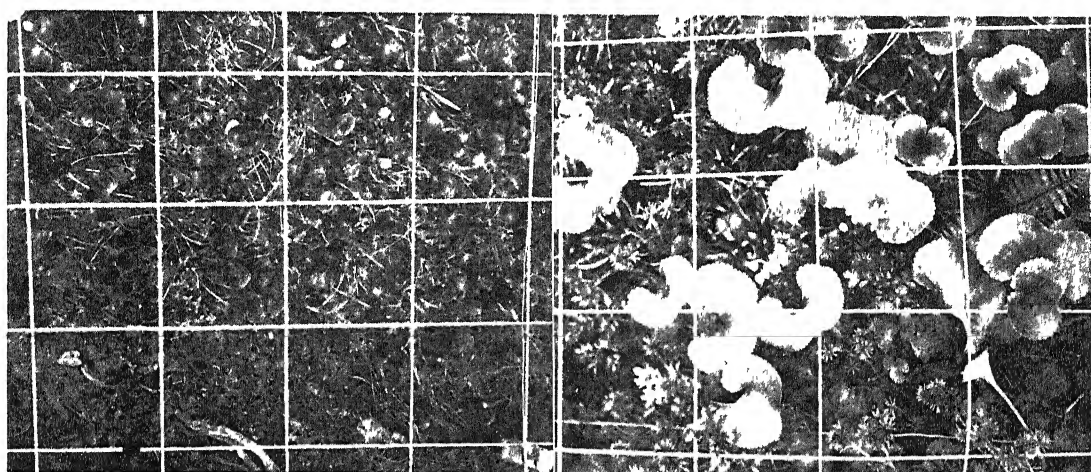
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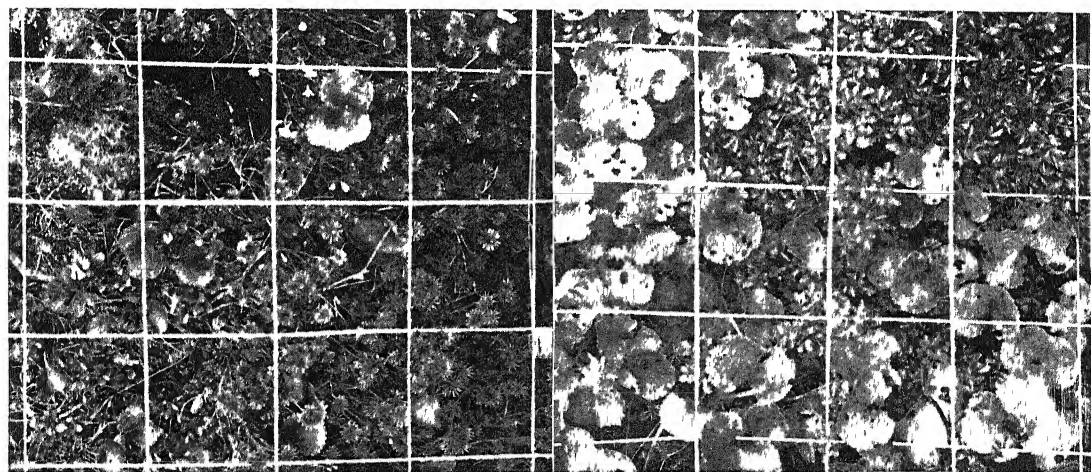
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THE COLONY OF THE LITTORINA: *LITTORIVAGA BREVICULA* (PHILLIPPI)¹⁾

By

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(With five text-figures)

(Received September 25, 1934)

INTRODUCTION

Littorivaga brevicula (PHILLIPPI) is widely distributed in Mutsu Bay and is found forming a colony on a rock, at times as large a number as more than 2000 individuals are found within a colony. Formerly, I have studied on the colony of the limpet, *Acmaea dorsuosa* GOULD, (N. ABE, 1933) and it was thought desirable to compare the colony formation of the limpet with that of *Littorivaga*. With this idea just stated the next observations and experiments were carried out at the Asamushi Marine Biological Station.

Before going further I wish to express my sincere thanks to Prof. SHINKISHI HATAI for his kind advice to my studies and correction of the treaties. On the identification of the species, I am indebted to Mr. TOKUBEI KURODA, whom I wish to express my hearty thanks.

I. DISTRIBUTION OF *LITTORIVAGA*

Distribution of *Littorivaga brevicula* in the neighbourhood of the Station is shown in Fig. 1. Comparing the distribution of this gastropod to *Acmaea dorsuosa* GOULD²⁾ (N. ABE, 1931), the latter inhabits the rocks where surging waves wash more frequently than other rocks; while the former inhabits the rocks where waves splash less strongly. *Littorivaga* is chiefly found on little rocks on a pebbly shore and I have never seen these gastropods inhabiting a sandy shore.

MITSUKURI (1901), HASEMAN (1911) and many other investigators have already shown that the *Littorina* inhabits the littoral zone. In the neigh-

¹⁾ Contribution from the Marine Biological Station, Asamushi, Aomori-Ken. No. 115.

²⁾ According to kindness of Mr. IWAO TAKI, the specific name of this gastropod is changed to *Patelloida (Tectura) grata dorsuosa* (GOULD).

bourhood of this Station, this gastropod also inhabits the littoral zone, but there is seasonal changes in its habitat as will be shown in the later pages of this paper.

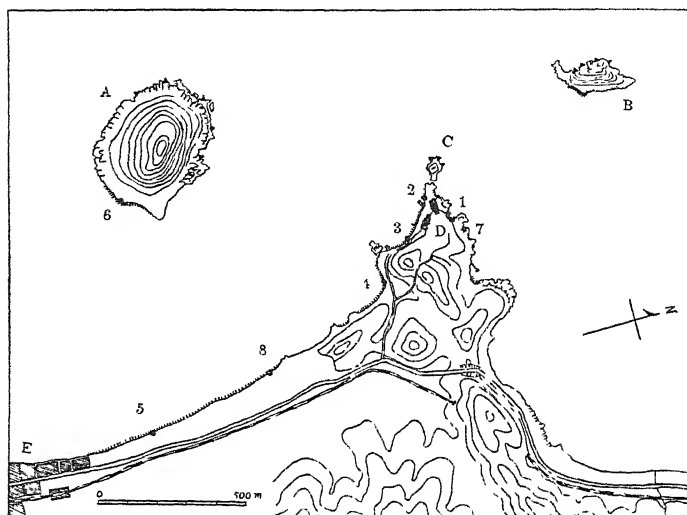


Fig. 1 Distributions of *Littorivaga brevicula*. Main habitat of *Littorivaga brevicula* Main habitat of *Acmaea dorsuosa* GOULD

A — Yunoshima, B — Gomishima, C — Hadakashima,
D — Marine Biological Station, E — Asamushi

i) *Distribution as a colony*. I have mainly paid my attention to the positions where the colonies are formed. At first I have determined five different positions at several localities where the colonies are formed and numbered 1 to 5 as are shown in Fig. 1. On the pebbly shore, the position of the colony was determined taking high tide line where short branches, straws and many other flotsams are gathered by waves as the origin, then measuring the distances sea-ward where the *Littorivaga* are inhabited. The results are shown in Table I.

In Table I, the seaward limit of habitat is about the same as the line of low-tide and the colony extends landwardly occupying usually one third of the entire littoral zone, but occasionally the size of the colony may occupy almost the middle of the littoral zone or the mean water level.

As to the position of the colony on little rocks or pebbles we find that it is formed on about the top of those as will be seen from Fig. 2.

TABLE I.

Positions of colonies of Littorivaga brevicula measured from high-water line.

(Oct 18th, 1933)

No of locality	Direction of the shoreline	Landward limit of the snails	Landward limit of colony.	Level along the maximum number of colonies	Seaward limit of colony	Seaward limit of the snails
1	NWW	0.1 m.	1.9 m	3.4 m	4.2 m	6.0 m.
2	SWW	0.2	1.7	2.8	3.8	5.7
		0.1	2.0	2.8	3.7	6.1
3	W	0.3	0.95	2.6	3.5	5.8
		0.1	1.2	2.4	3.7	5.7
4	S	0.5	1.0	2.3	2.5	4.1
		1.1	3.1	3.65	4.4	5.1
5	W	0.3	1.8	3.1	3.5	4.2
		0.5	0.95	2.5	3.1	4.7

W=west, N=north and S=south, etc



Fig. 2 Colonies of *Littorivaga brevicula* formed on a pebbly shore

The direction of the colonies were determined at 7 positions numbered 1 to 7 in Fig. 1, and the results are shown in Table II.

TABLE II.
Direction of colony.

No. of locality	Direction of the shore	Direction of colony									
		1	2	3	4	5	6	7	8	9	10
1	NWW	SE	SSE	SE	SE	SEE	SE	SEE	SEE		
2	SWW	NE	E	SE	E	E	E	E	E		
3	W	SE	SEE	E	SEE	E	E	E	E	E	NEE
4	S	N	NNE	N	N						
5	W	E	E	E	NEE	E	E	SEE	E	E	E
6	SE	NW	NW	NW	NNW	NNW	NNW	NW	NW		
7	N	S	S	S	S	S	SSE	SSE	SSE	S	S

E=east, W=west, N=north and S=south, etc

- Localities 6 and 7 were newly added

In Table II, it is seen clearly that the positions of the colony oppose the direction of the shore and thus it has no relation to the direction of sun light, but rather seems to me to be related to the direction of waves. Fig. 3 shows that the colonies are formed on the side of rock where the waves do not directly strike.



Fig 3 Colony of *Littorivaga* and waves. (photo Feb 17th, 1934).

I have observed another good example to show the relation of colony to waves. A square cement box of 1.2 metres stands at the seashore,

each face numbered from 1 to 8, and one side of the box faces the sea, namely to the west. On the 19th of October, 1933, the following distributions of *Littorivaga* were found (Table III).

TABLE III.
Distribution of Littorivaga brevicula on a cement box.

Direction of the face.	Colony			Defused inds		Other animals.
	No of colony	No of inds	Position	Nos	Position	
Outer sides						
W	1	4	about in the centre	73	lower half part	<i>Acmea dorsuosa</i> 1
S	10	540	lower east part	90	uniformly over the face	0
	3	32	upper edge			
E	11	1720	lower one third part	35	about near the margin	0
N	22	1710	lower one third part	27	uniformly over the face	0
Inner sides						
E	2	30	upper edge	17	uniformly	0
NE	1	50	upper corner	1	upper part	0
N	0	0	—	15	uniformly	0
NW	1	40	lower corner	0	—	0
W	0	0	—	21	uniformly	0
SW	2	200	lower corner	0	—	0
S	0	0	—	18	upper east part	0
SE	1	23	upper corner	3	lower corner	0

W=west, S=south, E=east and N=north, etc

In Table III, it is seen that the maximum number of snails are found on the outer face, which is directed east namely, land-ward. One of the outer faces, which is directed north, has an irregular surface and many individuals were found in these hollowed places.

ii) *Relation of the habitat to wetness.* A stone fence of 64 metres in length and about 6 metres in height stands along the shore line in front

of the Aquarium, and from it four earth pipes for drainage open at this fence, they are 15 to 17 metres apart. The height of the opening of the pipe is 1.5 metres from the level of high-water marks. From these pipes water is drained to the sea from the end of April to the end of November. I have measured the salinity of water from the pipe on the 14th of October and it was found that pipe No. 1 contained freshwater only, pipe No. 2 seawater only, pipe No. 3, 96% of seawater mixed with 4% of freshwater and pipe No. 4 91% of seawater mixed with 9% of freshwater.

At the lower side of the openings where the fence was wet, many *Littorivaga* are inhabited through the summer season, though the level of the pipes is about 2.2 or 2.3 metres higher than the ordinary level of colony. The observations made on the 20th of October, showed the following modes of distribution of *Littorivaga*.

- No 1 (freshwater only). Green algae covers the wet part by water drip, but *Littorivaga* is found neither on the green part nor at the mouth of the pipe, though several individuals are found on the boundary line of wet and dry part, the height of which is less than 60 cm. from the level of high-water marks
- No 2 (seawater only). Many *Littorivaga* were found in every place wet by the water, and 20 colonies were also found. 2 colonies composed of 20 individuals in one and 30 in the other were found within the pipe along the edge
- No 3 (seawater 96‰): About 23 colonies and many individuals were found scattered on the wet place. 3 colonies with about 20 individuals in each were found within the pipe along the margin.
- No 4 (seawater 91‰): About 16 colonies and many individuals were found scattered on the wet place. One colony with 7 individuals was also found within the pipe.

The above facts show clearly that the habitat *Littorivaga* is not limited to the littoral zone but may form at as high a place as about 2 metres than the ordinary colony level, if the rock is wet by seawater.

No other species of gastropod were found with *Littorivaga brevicula* with an exception of *Ligia (Ligyda) exotica* (ROUX) which was occasional found on the inner side of the pipe. But on the drier place of the bank on the level of the pipe, a small gastropod, *Littorivaga mullegrana* (PHILIPPI), is found. This gastropod seems to live, forming colonies only on a place higher than high-water marks throughout the year, the largest colony, so far noticed contained about 66 individuals mainly in the hollowed places as is shown in Fig. 4.

On the 9th of April, 1933, 4590 individuals were counted on the fence within 10 metres in breadth and about 2 metres above the water level. The vertical distribution of them was as follows:

-0.4	-0.2	0	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8 metres
0	?	394	586	616	740	613	683	627	331	0	

Where, 0 shows the level of high-water marks or the upper limit of *Chthamalus challenger* HOEK association, and — sign indicates below the high-water marks.

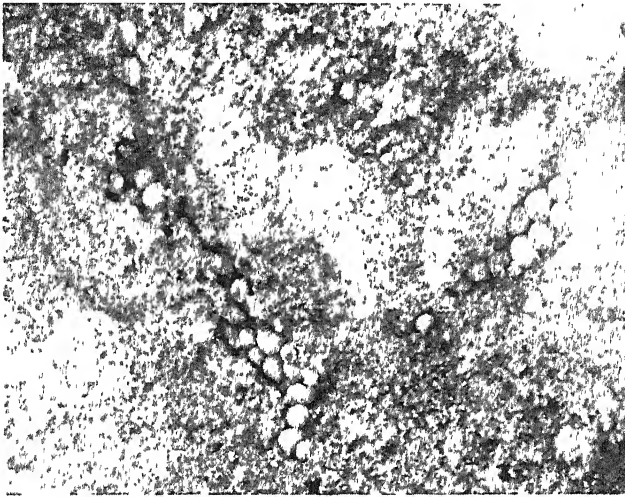


Fig 4 Colony of *Littorivaga millegrana* formed on a stone bank
(photo March 19th, 1934)

It seems very interesting, so far as my observations during one year period go, that the position of the colony of this gastropod did not show noticeable change throughout the year, and thus differs from the colonies of *Acmaea dorsuosa* GOULD or *Littorivaga brevicula* (PHILLIPPI). And I have never seen *Littorivaga millegrana* creeping in the field.

iii) *Change of colony.* The positions of the colonies did not show remarkable change in the days of autumn, though heavy waves splashed the rock or the pebbly-shore.

From the night of the 6th of November till the noon of the next day, a hurricane of 23 metres per second blew and the pebbly-shore was very much disturbed by heavy crushing waves. On the 9th, it was found that the colonies of *Littorivaga* were very much disturbed as stated below.

No 1. Colony was not found at all, and only two individuals were found on a little rock on the shore of about 14 metres breadth

- No 2: No colony and no individual were found
 No 3 No colony and no individual were found on the pebbly shore, but one colony with smaller sized individuals was found in a hollowed place of a rock.
 No 4 No colony was found, but several large individuals were found on the rock which stands in the water lower than the low-tide marks
 No 5 No colonies and no individuals were found

The positions from No. 1 to No. 5 are indicated in Fig. 1. Colonies formed in or around the earth pipe of draining water showed the following changes.

- No. 1 Water was stopped, and no individuals were found
 No 2 Water was still running but much reduced in its volume. Only 3 colonies with about 40 individuals in all were found in the mouth of the pipe and no individuals were found scattered
 No. 3 Water was not running One colony with 7 individuals alone was found
 No 4. Water was not running No individuals were found.

Continued observation during the next 10 days after the hurricane showed that neither colony was there formed nor any individuals came out. On the 9th of December, I have found large individuals on the shore though no colony was found on the pebbly-shore. On the later days, the number of *Littorvaga* gradually increased and a colony was found also.

The positions of newly formed colonies on the 16th of January, 1934 and on the 20th of February are shown in Table IV.

TABLE IV.

Positions of colonies of Littorvaga brevicula in winter, measured from high-water mark line

Date	No of locality	Land ward limit of the snails.	Land ward limit of colony of smaller snails.	Level along the maximum number of colony of smaller snails	Sea ward limit of the smaller snails	Land ward limit of colony of larger snails	Level along the maximum number of colony of larger snails.	Sea ward limit of colony of larger snails.	Sea ward limit of the snails
Jan. 16th	1	3.3 m.	4.3 m	4.8 m.	5.4 m.	5.8 m	7.0 m.	8.0 m.	8.0 m.
	2	4.0	4.0	4.6	5.8	5.9	7.4	7.8	7.8
Feb. 20th.	1	1.0	2.8		4.1	4.2	4.9	5.7	7.1
	2	2.2		3.0	4.8	5.1	5.8	7.3	7.3

In Table IV, we can see that *Littorina* retreated to a level lower than that of October, and the maximum level of the colonies of larger individuals retreated about 2 or 3 metres lower than former times. There

were found many individuals on sea weeds, *Sargassum Thunbergii* (KUNTZE) OKAM., *Colpomenie sinuosa* DERB. et SOL., *Scytosiphon lomentarius* (LYNGB.) J. Ag., *Chorda Filum* (L.), LAMOUR., and *Heterochordaria abietina* (RUPR.) S. et G.¹⁾ And some *Littorivaga* were feeding on those.

Those colonies formed in the mouth of the earth pipe, were no more to be found, and not even one individual.

II. EXPERIMENTS ON COLONY FORMATION

Littorivaga brevicula (PHILLIPPI) forms a colony in the room as in the natural habitat. So I have studied on the colony formation in the room wishing to find out some factors related to the colony formation and to compare it with the colony formation of *Acmaea dorsuosa* under similar conditions.

a) Method of experiment.

A middle sized cylindrical glass vessel, diametre 30 cm. and height 25 cm., was filled with sea-water up to 15 cm. The water is constantly running. The wall of the mouth was eight sected, and numbered from 1 to 8. The glass vessel is placed in the room, where diffused sun light enters freely from the south window, while the light from north, east and west were shut by a black curtain. The section No. 1 of the vessel is directed east-ward, No. 3 to the north, No. 5 to the west and No. 7 to the south.

On the experiment, individuals were placed on the centre part of the bottom of the vessel and on the next morning, the positions of the gastropods were marked by water colour from outside of the glass vessel. These marks were printed on a plotting-paper and subsequently their positions were carefully determined. All the materials employed were either collected from one colony or from the nearest colonies. The observations were continued from the 6th of November to the end of March.

b) Results of the experiments.

i) *The materials changed every day.* *Littorivaga* were collected from the rocky shore near the Station, and new materials were used every day. The results are shown in Table V.

¹⁾ On the identification of these sea-weeds, I am greatly indebted to Mr KÔGORÔ ABE. Here I wish to express my sincere thanks to him.

TABLE V.

Colony formation of Littorivaga brevicula on the individuals changed every day.

Date	Sections																
	1	1-2	2	2-3	3	3-4	4	4-5	5	5-6	6	6-7	7	7-8	8	8-1	
Nov. 6			4		(8)	65											
7	(4)			5(2)	30				(2)		(11)					3	
8											(8)						
9	85					7		2, 3		2	(11)				2	3	
10				3	39	2				(10)							
11																	
12					38						(5)						
13	(3)			2					(6)								
14	(4)				10(9)							—(48)—	5				
15	(3)												(70)				
16					4(11)							(14)					
17			5		(10)						6		3				
18			6		(2, 7						(3)						
19				7	(12) 21	2											
20	(3)				7, 21)						(6)			(2)			
21					13(15)						4(5)		(19)				
22				4	(21)	8		2			6		(14)			4	
23	(6)				(16)									6 (21)	(2)		
24	(7)			(9)						(2)			2(21)	2			
25	7			11	(2)									2(38)		7	
Total	Wall	12	0	15	32	169	84	0	7	0	2	16	0	10	8	2	17
	Base	(38)	0	0	(11)	(127)	0	0	0	(8)	(12)	(49)	(62)	(124)	(53)	(2)	0

Numbers in () stand for the colony formed on the base of the vessel and the other numbers indicate those on the wall

In Table V, it becomes clear that the positions where the colonies are formed are mainly near section No. 3, but the colonies on the bottom were found at section Nos. 3, 6 and 7.

ii) *The same materials used continuously without renewing.* In the former experiment, *Littorivaga* formed a colony in the darkest place in the vessel. But I wished to know whether or not the positions of colony changes. To test this point, about 150 individuals were placed on the

bottom and observation was continued during about 2 months beginning at the 10th of November. The results are shown in Table VI.

TABLE VI.

Colony formation of Littorivaga brevicula on the individuals observed continuously.

Date	Sections															
	1	1-2	2	2-3	3	3-4	4	4-5	5	5-6	6	6-7	7	7-8	8	8-1
Dec																
17	(7)				81		9		18		2, 16		8		(5)	
18																
19	(8)(2)									(31)	92				(26)	
20	(5)									(35)		35		(53)		
21	(4)								(12)				41		(48)	
22	(6)												(8)	52	(30)	
23	(6)												19	4	77	
24																
25	(11)													6	(99)	
26	(11)				(3)									8	(98)	
Jan.																
15	(3)	(39)			(6)							15	(6)		(16)	
16	(4)			2	(5)				4			3, 2	5, 3		(52)	
17	(5)	(33)			(5)		(5)					(3)	15, 2		(18)	
18	(4)	(32)			7	2	(3)					3, 17	3, 3		(14)	
19	(2)	(32)			(2)			(3)		(2)(4)			29		(7)	(3)
20	(2)	(38)					5		(2)(3)	(4)			18(2)	7	(6)	
21																
22	(4)	(41)					5			(4)(5)		10	9	(6)		
23	(6)	(44)	3	(9)	(2)		(6)			(4)		(2)	(2)	(9)		
Total	Wall	0	0	3	2	7	2	10	0	0	0	33	57	7	0	0
	Base	(30)	(259)	0	(9)	(20)	0	(14)	(3)	(9)	(23)	0	(22)	(13)	(15)	(61)
																(55)

Numbers in () mean that the colony formed on the base of the vessel, and the other numbers mean the ones on the wall

In Table VI, it is shown that the colony with the largest number was formed in the darkest place on the 17th of December, but on the next day, most of the individuals assembled at the section No. 6 or No. 7, the brightest place in the vessel. In the following 70 days, the position of the colony showed no further remarkable change, excepting the number

of individuals in the colony showed slight daily fluctuation.

iii) *Relation of colony to the water surface.* Some individuals within a colony stayed in the water, some on the line of water surface and still some others above water level. If the level below 0.5 cm. and 0.5 under the water surface is taken as 0 level and the next one centimetre above

TABLE VII.

Relation of colony of Littorivaga brevicula to the water surface.

Date	Vessel	Section	Level												
			-6	-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5	+6
Nov. 10	A	3					3	9	11	7	8	1			
	B	4							5	2	1				
14	A	3			1	3	4	2							
	B	1-8				2	4	6	7	4					
16	A	3						1	3						
	B	1							4	2	2				
	B	3							1	2	2				
17	A	2						1	4						
	A	7						1	1	1					
	B	3						2	3	3	3				
18	A	2						1	5						
	A	3						1	5	1					
	B	3					1	4	6	3	2				
19	A	3			1	1	1	7	5	5	1				
	B	3			1	3	3	7	11	5	1				
20	A	3					1	2	4						
	B	3			1	3	4	6	7	1					
21	A	3						1	6	3	2	1			
	A	4							3	1					
22	A	2-3									1	2	1		
	A	3-4										1	5	2	
	A	6									1	2	2	1	
	A	1-8							3	1					
23	A	7-8						2	4						
25	A	2-3					1	3	3	3	1				
Total numbers			0	0	4	12	22	56	101	44	25	7	8	3	0

In the column, A and B stand for two kinds of vessels but of about the same size.

as +1 level and one centimetre under as -1 level, etc, the distribution of those individuals in each level is shown in Table VII, and also in Fig. 5.

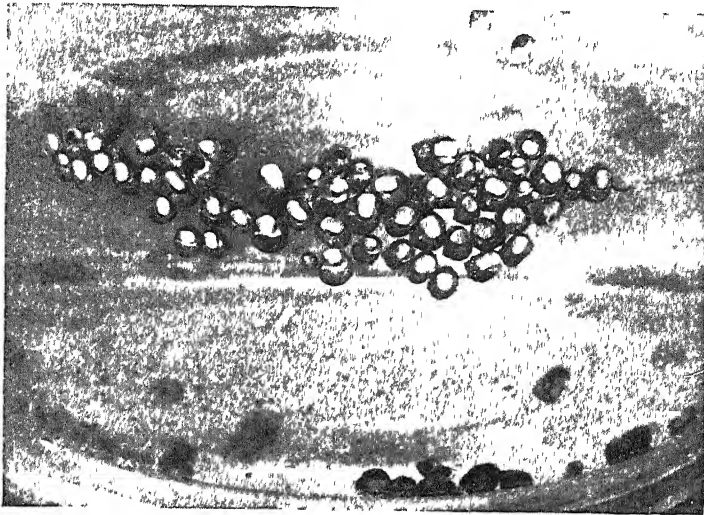


Fig 5 Colonies formed in the glass vessel (photo. Feb 11th, 1934).

In Table VIII, similar observations on the colony of *Acmaea dorsuosa* as that of *Littorivaga* are shown. In the case of *Acmaea*, the distance of each level was 2 centimetres owing to the greater shell length. (One centimetre in *Littorivaga* and 2 centimetre in *Acmaea* are about the mean shell length).

Both in *Littorivaga* and in *Acmaea*, those individuals which occupy the 0 level are more numerous than any other levels. And the ratios of number of individuals in the water to the air are about 1 : 0.93 in *Littorivaga* and 1 : 1.50 in *Acmaea* respectively. So it may be said that *Acmaea dorsuosa* shows the stronger tendency to assemble in the air than in the water, while *Littorivaga* shows about the same intensity to assemble both in water and in the air. These tendencies just found are well seen in their distribution in the field, but in the case of *Acmaea dorsuosa*, the nature of formation of colony in the room is somewhat different than in the field, in forming at a level always above high water marks.

iv) *Change of nature of colony formation in the room.* As was mentioned already, the number of individuals within the colonies changed daily as was shown in Table VI. For instance the colonies formed on the glass wall, contained 134 individuals on Dec. 17th, but day by day

TABLE VIII.

Relation of colony of Acmaea dorsuosa to the water surface.

Date	Section	Levels								
		-4	-3	-2	-1	0	+1	+2	+3	+4
Sept 19	2			1	1	2				
"	3					2	1			
"	8					2	4			
24	1			2	3	4	1			
"	5					2	2			
25	1			2	3	3	1			
"	5					1	3			
26	1		1	2	3	3	1			
"	5					1	2			
27	1		1	3	3	3	1			
Oct 19	1			2	2	3	2			
20	7				2	3	3	1		
21	5					4	1			
"	7				1	3	2	1		
22	7					4	2	1		
23	5					3	2			
24	5					3	2	1		
25	5					3	3			
26	7				3	2	1			
27	1			1	2	3	2			
"	7				2	2				
28	5				2	2	2			
30	5					3	4	1		
Nov 1	5					4	3	1		
12	5					3	4	2		
18	5					2	4	3		
Total numbers		0	2	13	27	70	53	11	0	0

the number decreased and on Dec. 25, only 6 individuals were found though they showed no further decrease till the beginning of January, 1934. The number however began again to increase till it reached to 29 on Jan. 19, but then decreased again to 3 on Jan. 23. After Jan. 23 it showed a slight but steady increase; that is 22 on Jan. 25, 30 on Jan. 30, 41 on Feb. 1 and 45 on Feb. 7.

It was further noted that the numbers show fluctuations not merely

by day but even in every hour so far as it was observed from 9 o'clock in the morning to 6 o'clock in the evening. As for instance on the 7th of February, it was found in one colony 38 at 9:0 am.; 38, 10:0 am.; 39, 11:0 am.; 37, 12:0 am.; 39, 1:0 pm.; 38, 2:0 pm.; 36, 3:0 pm.; 38, 4:0 pm.; 40, 5:0 pm.; 40, 6:0 pm.

v) *Colony formation at the end of March.* The colony formation of *Littorivaga brevicula* was again observed at the end of March using the same method as the previous experiment and also using new materials every day. The results are shown in Table IX.

TABLE IX.

Colony formation of Littorivaga brevicula at the end of March.

Date		Sections															
		1	1-2	2	2-3	3	3-4	4	4-5	5	5-6	6	6-7	7	7-8	8	8-1
March	21					2						106				2	
	24	(3)		3		2		27			9	20		8		2	26
	29											115		2	(3)	18	
	30	5(4)					2		15		73			(4)	(9)	33	
	31	(4)											60	(68)			
April	1		3		5		2				3	(3)	96	(11)	(2)	15	
Total	Wall	5	3	3	5	4	4	27	15	0	85	125	262	10	0	68	0
	Base	(11)	0	0	0	0	0	0	0	0	0	(3)	0	(83)	14	2	26

Numbers in () indicate the colony formed on the base of the vessel and the other numbers indicate those on the wall.

In Table IX, we see that the positions where the colonies are formed are mainly on the brighter side of the vessel, quite opposite to the results previously observed in November.

III. GENERAL CONSIDERATION

Majority of investigators who studied on the behavior of periwinkles, *Littorina*, gave special attention to the rhythmical movement of the snails in relation to tide and only few on the colony of the snails.

1. HASEMAN (1907) who studied on the habitat of *Littorina rudis* and *L. palliata* stated that "The variation in the temperature along the coast did not appear to affect the distribution of *Littorina*. Snails were subjected to much greater changes of temperature than occur along the

coast and no observable effects were detected."

But as for *Littorivaga brevicula*, the seasonal variation in the habitat is evident: in autumn, both larger and smaller individuals are found one foot lower than low-water marks as HASEMAN already stated, though the majority of them are found on the level of mean-water level. But in the beginning of winter and when snow begins to fall, the snails begin to move towards a much lower level and many individuals formerly found on the rock near high-water marks gradually disappear and ultimately none are to be seen. It is interesting to note that the habitat of larger and smaller individuals are clearly separated and smaller individuals occupy the level somewhat higher than that occupied by the larger. Furthermore both in *Littorivaga brevicula* and in *Acmaea dorsuosa* (N. ABE, 1933) the smaller individuals show a less degree of seasonal variation than that exhibited by the larger ones. In early spring, the snails begin to move towards the upper level and some of them are found on the rock on the level of high-water marks.

2. MITSUKURI (1901) stated that, "*Littorina exigua* are in nature scattered over rocks, because there are unevenness in rocks which provide them with holes and crevices to settle down in." I have also noted in the shore near the Station, that the snails are found in scattered state settling down in the hollowed clay-slate made by a boring shell. At the same time I found many colonies which are formed on one part of the rock surface which is very uneven.

On the pebbly-shore, the positions where colonies are formed are about near the top of the stone. If the opinion of HASEMAN that the movement of *Littorina* is very much related to the surface film of water was accepted, we may interpret our case just stated that the colony on the top of the stone was formed pursued by water film at increasing tide, but difficulty arose since the colony remains on the stone though the water film retreats at decreasing tide. I have already stated that in the room the colony is also formed though the surface film of water was kept at a constant height. It seems therefore that the colony is not formed in the field by merely the movement of surface film of water, though it plays a very important role on the movement of snails as MITSUKURI and HASEMAN have already found, and as I have also noted with *Acmaea dorsuosa* and with *Batillaria mutiformis* (N. ABE 1933, '34).

3. Both MITSUKURI, and HASEMAN have already noted the importance of wetness concerning the movement of the snail, and I have also found that the colony is formed even on the level of 1.5 metres higher than

the normal level when the passage was wet by sea-water.

4. As it was already stated the snail forms the colony on the position which is directed landward. According to MITSUKURI, "*Littorina exigua* shows a strong negative phototaxis under ordinary circumstances... This property enables the mollusc to creep up from the sea towards the higher level which corresponds in most cases with land, but in cases of detached rocks may be away from the general mass of land." It follows then that the colony formed on the landward is by the result of negative phototropism. It seems to me more natural to consider that many individuals assembled on the side of landward for the sake of avoiding the direct splashing wave.

5. The colony formation by the same individuals observed continuously in the room showed negative phototropic nature in November and in early December, which in turn changed to positive on about the 19th of December. At first I thought that this change of phototropic nature may have occurred according either to the change of oxygen content of the water or by other external factors. But the formation of the colony on the brighter side at the end of March seems to indicate that the change of phototropic nature can not be a forced result of abnormal environment in the room.

The change of phototropic nature has already been noticed by many investigators. MITSUKURI (1901) finds that when desiccated, *Littorina* became positively phototactic, and when wet, turned negatively phototactic. G. BOHN (1904) considers that phototropic nature of *Littorina* is changed in relation to fortnightly (spring and neap) rhythm in tide (Referred from M. W. MORSE, '09-'10). MORSE (1909-'10) who studied on *Littorina littorea*, *Littorina rudis*, and *Ilyanassa obsoleta* says that, "During the days of June, they were, as a rule, negatively phototactic, and as night approached, they became positively phototactic. However, after July 18, the preponderance of positive phototaxis during the day was very noticeable. This period of transition corresponded to the time of change from spring to neap tide, during which the specimens out on the rock were exhibiting a corresponding change in phototaxis, for the water did not reach them."

But in my case, *Littorivaga* did not show such a daily change in phototropic nature in the vessel but seasonal change was found. I think more an exact experiment in the field is necessary to test this before any definite statement can be made on this point.

SUMMARY

1. *Littorivaga brevicula* (PHILLIPPI) inhabits the littoral zone, but shows seasonal variation in its habitat. The habitat of smaller individuals

is found at a somewhat higher level than that occupied by larger individuals in winter.

2. Stormy waves give very destructive influence to the habitat of *Littorivaga brevicula* though not so much to that of *Littorivaga millegrana* (PHILLIPPI).

3. Colony of *Littorivaga brevicula* is formed mainly near the top of a pebble or in the hollowed place of a rock. Most of the colonies are formed on the side directed landward, suggesting that the snails avoid the direct splashing waves. No definite relation to the direction of sunlight was found.

4. When the rock is wet by sea-water, the colony may be formed at as high a level as 1.5 metres from the normal position.

5. *Littorivaga millegrana* forms a colony always above the high-water marks, and seasonal variation in its habitat was not observed.

6. *Littorivaga brevicula* forms a colony also in the laboratory. In November and in early December, a colony is found on the darker side of the vessel, but at the end of March, on the brighter side of the vessel.

7. Relation of the colony of *Littorivaga brevicula* to the water surface shows a slight daily change but most of the individuals within the colony are found on the level of the water surface and the ratio between the individuals found in the air and in the water is about 1:0.95 in autumn and in winter.

8. Relation of a colony of *Littorivaga brevicula* to the water surface is more changeable when compared with *Acmaea dorsuosa*, the individuals which are found on the level of the water surface while the ratio between the individuals found in the water and in the air is about 1.0:1.5.

REFERENCES

- ABE, NOBORU 1931. Ecological observations on *Acmaea dorsuosa* GOULD. Sci. Rep. Tôhoku Imp. Univ., Biol., Vol. VI, No. 3, pp. 403-427.
- ABE, NOBORU 1933. The colony of the limpet (*Acmaea dorsuosa* GOULD). Ibid. Vol. VIII, No. 2, pp. 169-187.
- ABE, NOBORU 1934. Ecological observation on *Batillaria multiformis* (LISCHEKE). Ibid. Vol. VIII, No. 4, pp. 383-398.
- BOHN, GEORGES 1904. Compt. rend. d l'Acad. des sciences, 1904, cxxxix, 610, 646.
- HASEMAN, J. D. 1911. The rhythmical movement of *Littorina litorea synchronous* with ocean tides. Biol. Bull., Vol. XXI, No. 2, pp. 113-121.
- MITSUKURI, K. 1901. Negative phototaxis and other properties of *Littorina* as factors in determining its habitat. Annot. Zool. Japonenses, Vol. IV, Part 1, pp. 1-19.
- MORSÉ, MAX WITHROW, 1909-10. Alleged rhythm in phototaxis synchronous with ocean tides. Proc. Soc. Exp. Biol. & Med., Vol. 7, p. 145.

NOTES ON THE BEHAVIOR OF *NERITA JAPONICA* DUNKER

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(With eleven figures)

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Nerita japonica DUNKER is commonly found in the neighbourhood of the Institute attached to rocks especially at the high water mark. The shell of *Nerita japonica* is oval in shape with irregular marking on its surface and has a hard operculum. The purpose of the present study is to analyse the ecological characteristics of this animal.

The observations and the experiments have been done at the Mitsui Institute of Marine Biology. Here I wish to express my heartfelt thanks to Prof. S. HATAI and Dr. N. ABE for their valuable criticism.

GENERAL OBSERVATIONS

Habitat

Nerita japonica DUNKER is found, mostly in groups, in crevices or in holes in the rock. On the vertical or horizontal rock-surfaces where the sun directly shines all day, these animals are found in small numbers and never seen to form groups. *Nerita japonica* does not occur in dry places above high-water neaps which are exposed to direct sunlight during the whole or a long portion of the day. In holes, where small tide pools are formed, the animals are grouped on the wet or shaded sides of the rocks, but a few animals were in the water (Fig. 1).

Around or in the groups of *Nerita japonica* are found *Littorwaga brevicula* (PHILIPPI), *L. millegrana* (PHILIPPI), and *Batillaria multiformis* (LISCHKE). When the sea was high, *Monodonta labio*

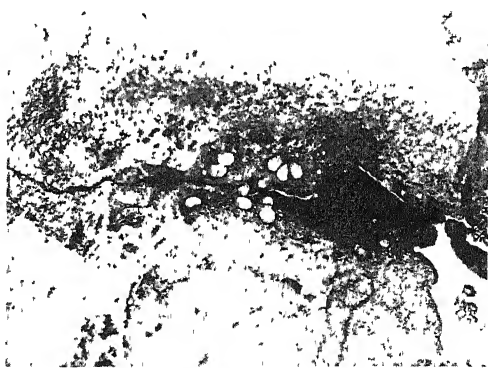


Fig 1. A group of *Nerita japonica* Reduced to 1/6 natural size Photographed on July 12, 1933.

(LINNÉ) and *M. neritoides* (PHILIPPI) are often found in their groups.¹⁾

Habits

Nerita japonica DUNKER moves actively while the rocks are wet, but when the sun shines directly upon them and the rocks become dry, they conceal themselves in the shady places and do not move until the rocks become wet again. As their habitat are at the high-water mark, they are washed with the waves. Therefore when the sea becomes high, they move actively, but when it becomes low, they do not. At high-water in a damp situation or in situations not exposed to direct sunlight some individuals can usually be found creeping and feeding, but in dry situations at high-water which are exposed to sunlight the animals are rarely seen on the move except in damp or wet weather. But if the wind blows strong and the waves splash along the shore, or when it is rainy, they

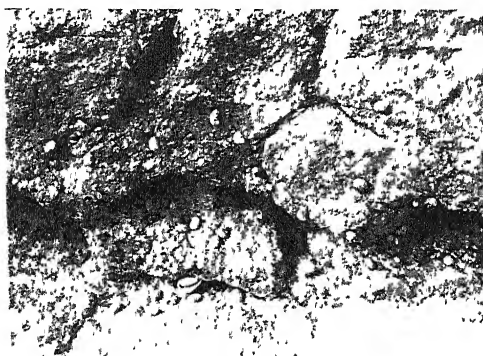


Fig 2. A group of *Nerita japonica*, showing the creeping on the wet rock after a rain. Reduced to ca. 1/6 natural size. Photographed on July 16, 1933.

creep along the wet places apart from the crevice or the hole of rock even though the sea is low (Fig. 2). Often I observed that some animals moved landward when the rock was wet, and stopped in some holes. Such animals may stay for several days in the same spot if the weather continues to be fine.

Almost all *Nerita japonica* are found on the rocks and very few or very rarely on the sand, though they are able to creep very slowly

on the wet sand. The animals found on the sand might have crept down from the rock either by the waves or by any accidents.

The velocity of locomotion of *Nerita japonica* is about 7 centimeter per minute on the rock-surface but on the sand it is about 2-3 centimeter per minute.

On the type of the movement of *Nerita*, PARKER ('11) already observed.

¹⁾On the identification of the species name of the shells above quoted, I am much indebted to Dr. T. KURODA in the Geological Institute, Kyoto Imperial University, to whom I wish to express my sincere thanks.

He mentions in *Nerita tessellata*, "the wave begins anteriorly as a single wave whereupon it breaks and passes down right and left sides of the foot to unite as one wave again at the posterior margin." In this species, *Nerita japonica*, the mode of the locomotion was quite identical with *N. tessellata*. This mode of locomotion belongs to the so-called "Ditaxic" of "Retrograde type".

Nerita japonica crawls in a forward direction or somewhat side-way, but never moves backward, as PARKER mentioned ('11), even when disturbed by sticking with a needle. When it is suddenly disturbed by needle-sticking or by any chemical agents, it turns and changes its direction immediately or shuts its operculum and ceases locomotion. When this animal was placed up-side-down on the flat bottom, the animal could easily turn itself right side up, even though the bottom consisted of sand or a glass plate.

10 individuals from one group were numbered on the shell with enamel and placed at definite places where they may be washed with only high-water of the spring tide, and their new places were marked after every 24 hours as shown in Figures 3 and 4 (Table I and II).

The following facts may be seen in Figures 3, 4; Tables I and II;

- 1) *Nerita japonica* moves about 2 meters a day.
- 2) The animal creeps along the slit of the rock and stops about the high-water mark of the neaps.

TABLE I.
Locomotion of Nerita japonica in nature.

Temp	C	Atmos.			
		Water	25 0	27.8	24.0
			21.8	23.3	22.5
Date		16/VI	17/VI		18/VI
Shell No			Moved dist for 24 hours	Dist. of animals from the first start	Moved distance for 24 hours
			Distance from the start point	from the first start	Dist. of animals from the first start
1			219 cm.	32 cm.	231 cm.
2			27	67	75
3			209	20	195
4			159	44	175
5			162	57	190
6			146	63	170
7			161	49	179
8			25	150	225
9			224	292	236
10			137	54	195

TABLE II.
Locomotion of Nerita japonica in nature.

Temp °C.	Atmos.	26.0	26.0	24.2	
	Water	22.4	22.5	22.0	
Date	11/VII	12/VII		13/VII	
Shell No.	Moved dist. for 24 hours Distance from the start point	Moved distance for 24 hours	Dist of animals from the first start	Moved distance for 24 hours	Dist. of animals from the first start
1	78 cm	0 cm	78 cm	0 cm.	78 cm.
2	214	0	214	0	214
3	96	128	202	7	195
4	125	0	125	22	147
5	180	66	246	0	246
6	134	0	134	0	134
7	151	0	151	7	158
8	122	157	33	94	97
9	182	12	170	0	170
10	181	34	215	0	215

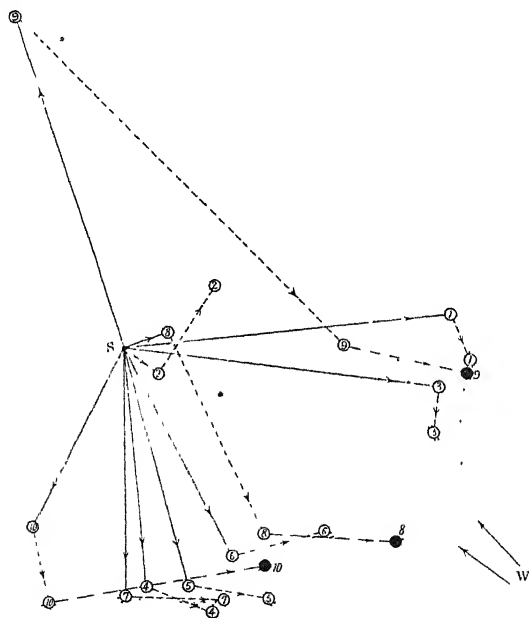


Fig. 3. Diagrammatic tracing the locomotion in nature in Table I. Dotted lines indicate the crevices of rocks. S, start point; W, the direction of sea water current.

—○ 16/VI, ----○ 17/VI, -.-.-● 18/VI.

3) The animal moves the longest distance in the first day. This fact may mean that the animal seeks a suitable habitat, since it does move no more after reaching a suitable place.

It has been known that the limpet has a home and returns to the "Scar", even after wandering some distance in search of food. ORTON ('29) says that *Patella vulgata* has a home but that it is not the final dwelling. ABE ('31) agrees with ORTON in *Acmaea dorsuosa*. I examined whether *Nerita japonica* has a home or not. With enamel I marked the shells of the animals of one group in one square meter (55 individuals) and every day counted their number. Some animals stayed for more than 20 days at one spot but others crept away and never came back. The number of the marked animals decreased day by day and, on the other hand, the no-marked ones increased. After half a month, I found half the total number

of the group to belong to the no-marked animals. The marked animals vacated the first place and were found mixed in other groups. Some of them crept away more than 10 meters after occupying the first place for 20 days. After a month from the beginning, almost all of the marked animals were not found at the first place and yet the number of animals of the group showed no decrease. So far as the period of my observation goes it appears that these animals have no definite home.

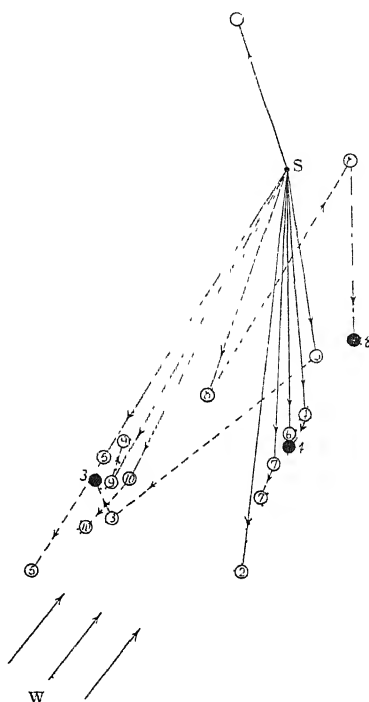


Fig 4 Diagrammatic tracing the locomotion in nature in Table II Dotted lines indicate the crevices of rocks S, start point, W, the direction of sea water current

· · · · · ○ 11/VII, - - - - - ○ 12/VII,
- . - . - ● 13/VII.

Duration of Life.

From the facts that *Nerita japonica* could move on the wet rocks, whether by the waves, or by the rain, and furthermore, that they could live on the rock which is exposed to the air or in the tide pool, as mentioned above, I attempted to determine how long these animals could live in water or could endure dryness.

Experiment I. In the water.

A. Distilled and Tap water. I prepared 4 glass bottles (350 cc.), into which 3 animals were put in each bottle. The bottle was filled with water and on it was placed a glass lid so as to refuse air space. The water was changed twice a day. When the animals were put in the water, they shut their operculum immediately and never moved. When the animals grew weak, some began to open their operculum. Occasionally I examined whether or not they still live by inserting a needle between the operculum and the shell. Some of them were returned to the running sea water to see if they would recover. When they did not show any reaction to the needle insertion and did not move again in normal sea water, I determined that they were dead. The results of these experiments are as follows: (Tables III and IV).

TABLE III.

Duration of life of Nerita japonica in distilled water
(Experiment I, A.)

Temp. °C	Atmos.	26.0	23.7	24.3	24.3
	(Water)	25.6	23.2	23.5	23.5
Hours after immersion					
Shell length in mm.		24	48	52	56
13.35		+	+	—	
13.5		+	+	—	
13.65		+	—		
13.85		+	+	—	
14.55		+	+	+	—
14.65		+	+	—	
14.8		+	+	+	—
15.1		+	+	+	—
15.3		+	+	—	
15.4		+	+	—	
15.5		+	+	—	
15.5		+	—		

+ means that the animal still survive and — indicates the death.

TABLE IV.
Duration of life of Nerita japonica in tap water
 (Experiment I, A)

Temp °C	{ Atmos	29.0	29.2	29.4	26.0	26.5
	{ Water	26.0	25.5	25.8	24.5	24.7
Hours after immersion		24	48	55	72	80
Shell length in mm						
13.4		+	+	—		
13.7		+	+	+	—	
14.0		+	+	+	—	
14.2		+	+	+	—	
14.3		+	+	—		
14.55		+	+	+	+	—
14.6		+	+	+	—	
15.3		+	+	+	—	
15.35		+	+	+	—	
15.4		+	+	+	—	
15.4		+	+	—		
16.0		+	+	+	—	

+ means that the animal still survive and — indicates the death

From the tables, we can see that *Nerita japonica* can live in distilled water for about 50 hours and in tap water for 3 days. The size of the animal has no correlation with the duration of life.

B. Diluted sea water. The sea water was diluted with tap water and their duration of life was examined as in experiment A. In this case also the water was changed twice a day.

- a 1/4 vol sea water+3/4 vols tap water 25% sea water.
 b 1/2 vol. sea water+1/2 vol. tap water .50% sea water

The results of these experiments are as follows (Table V). In the case of a, *Nerita japonica* can live for about 3.5 days and in the case of b for 4 days. In these cases also, the duration of life and the size of the animals has no correlation.

C. Running sea water and Control. In a glass bottle of 1,300 cc., 12 individuals (shell length 12.5–16.2 mm.) were placed and fed with running sea water. The animals move here and there in the bottle, but about 1.5 month later, their movements became weak and some of them fell to the bottom of the bottle up-side-down. I picked up such fallen animals and examined whether or not they live by inserting a needle as in the cases of experiments A and B. They could live in such running sea water for 2–3.5 months. Even the weakest ones could live for 57 days.

TABLE V.

*Duration of life of Nerita japonica in diluted sea water
(Experiment I, B.)*

	Temp °C	{ Atmos Water	26.0	23.7	26.8	27.1	27.0	28.3	28.6
			23.6	22.4	22.7	22.8	22.8	24.5	24.7
	Hours after immersion	Shell length in mm.	24	48	72	76	80	96	103
25% sea water	13.4	+	+	—					
	13.45	+	+	+	+	—			
	13.7	+	+	+	+				
	13.7	+	+	+	+	—			
	14.3	+	+	+	+	—			
	14.8	+	+	+	+	—			
	15.2	+	+	+	—				
	15.25	+	+	—					
	15.3	+	+	—					
	15.7	+	+	+	+	—			
	16.45	+	+	+	—				
17.9	+	+	+	+	—				
50% sea water	13.6	+	+	+	+	+	—		
	13.9	+	+	+	+	+	—		
	14.4	+	+	+	+	+	—		
	14.5	+	+	+	+	+	—		
	14.5	+	+	+	+	+	—		
	14.8	+	+	+	+	+	+	—	
	15.1	+	+	+	+	+	+	+	—
	15.5	+	+	+	+	+	+	+	—
	15.7	+	+	+	+	+	+	+	—
	16.05	+	+	+	+	+	+	+	—
	16.5	+	+	+	+	+	—		
	17.2	+	+	+	+	+	—		

+ means that the animal still survive and — indicates the death.

For the control, the same size glass bottles as in experiments A and B were used and the water was changed twice a day. The shell length of the materials were 11.8–15.8 mm. In normal sea water, the animals could live for about 1.5 months, excepting the one which survived for 2.5 months.

In these cases also the size of the animals were independent to the duration of life of the materials.

D. Concentrated sea water. When these animals are in the tide pool, the water evaporates gradually and the salinity of the water might rise somewhat. Therefore I tested how long they could live in such changed water.

The salinity of the sea water was increased by the addition of NaCl,

0.5%, 1%, 2%, and 4% respectively, then put the materials similarly in experiments A and B. In 0.5%, 1%, and 2% concentrated sea water the animals moved as in the normal sea water, but in the 4% concentrated sea water they shut their operculum from the beginning and never moved. In either cases, when the animals grew weak, they open their operculum and fell on the bottom of the bottle upside-down. The following tables (VI and VII) show the results of these experiments.

TABLE VI.

Duration of life of Nerita japonica in concentrated sea water
(Experiment I, D)

Water temperature 21.0~24.3°C

	Days after immersion	7	9	12	15	20	28	33
	Shell length in mm							
0.5% concentrated sea water	12.0	+	+	+	+	+	-	
	12.0	+	+	+	+	+	-	
	12.2	+	+	+	+	+	+	-
	12.5	+	+	+	+	-		
	13.1	+	+	+	+	+	+	-
	13.5	+	+	+	+	-		
	14.1	+	+	+	+	-		
	14.3	+	+	+	+	+	-	
	14.5	+	+	+	+	-		
	14.6	+	+	+	-			
	14.7	+	+	+	-			
	15.0	+	+	+	+	-		
1% concentrated sea water	11.2	+	+	+	-			
	11.65	+	+	+	+	+	-	
	12.35	+	+	+	-			
	13.0	+	+	+	-			
	14.0	+	+	-				
	14.0	+	+	+	+	-		
	14.0	+	-					
	14.2	-						
	14.3	+	+	+	+	+	-	
	14.6	+	-					
	14.9	+	+	-				
	15.1	+	-					

+ means that the animal still survive and - indicates the death.

Experiment II. In the Air.

A. I placed 15 individuals in a glass dish after the adhered water was wiped away and then placed in the room. The animals shut their operculum, not entirely but leaving a little opening. By inserting a needle through their slight opening, they shut their operculum hurriedly but after

TABLE VII.

*Duration of life of Nerita japonica in concentrated sea water
(Experiment I, D.)*

	Temp °C.	{Atmos. Water	28.5	28.3	28.3	27.1	27.6	28.0	28.7	29.0
			25.8	24.7	24.8	24.2	25.2	25.4	25.2	25.2
Hours after immersion										
Shell length in mm			24	40	48	64	72	90	114	136
2% concentrated sea water	12.65		+	+	+	+	+	+	+	—
	13.2		+	+	+	+	+	+	+	—
	13.5		+	+	+	+	+	—		
	13.6		+	+	+	+	+	+	—	
	13.7		+	+	+	+	+	—		
	13.85		+	+	+	+	+	—		
	13.9		+	+	+	+	+	—		
	14.3		+	+	+	+	+	+		
	14.4		+	+	+	+	+	+	—	
	15.0		+	+	+	+	+	+	—	
	15.1		+	+	+	+	+	—		
	15.7		+	+	+	+	+	—		
	12.3		+	+	—					
	12.4		+	+	+	—				
4% concentrated sea water	12.6		+	+	—					
	12.8		+	+	—					
	13.2		+	+	+	—				
	13.5		+	+	+	—				
	13.5		+	+	+	—				
	13.6		+	+	+	—				
	14.1		+	—						
	14.6		+	+	—					
	15.1		+	+	+	—				
	16.55		+	+	—					

+ means that the animal still survives and — indicates the death

several hours they again opened their operculum a little. As the day passed, the animals shut their operculum tightly but did not cement the operculum by any mucus. They could live for about one month in such a condition as shown in Table VIII.

B. 20 individuals were placed on a stone which was entirely dry and were exposed in a sunny place. After 4 hours, I returned the animals to the running sea water but all were dead. Then I gathered 150 animals (shell length 13–17 mm.) from the shore and repeated the same experiment. But in the present experiment, I picked up 20 animals at random after every 30 minutes and returned them to the sea water. After exposure for 30 minutes, all animals were alive but exposure for 2 hours kills nearly 60% of them. The result of this experiment is shown in Figure 5.

TABLE VIII.

Duration of life of Nerita japonica in the air
(Experiment II, A.)

Temperature: 23.0~28.5°C.

Days after Shell length in mm	26	28	30	32	35
12.3	+	+	—		
13.2	—				
13.4	+	—			
13.5	+				
14.3	+	—			
14.4	+	+	+	—	
14.6	+	+	—		
15.2	—				
15.2	+	+	+	+	—
15.4	+	+	+	—	
16.3	—				
17.1	—				

+ means that the animal still survive and — indicates the death

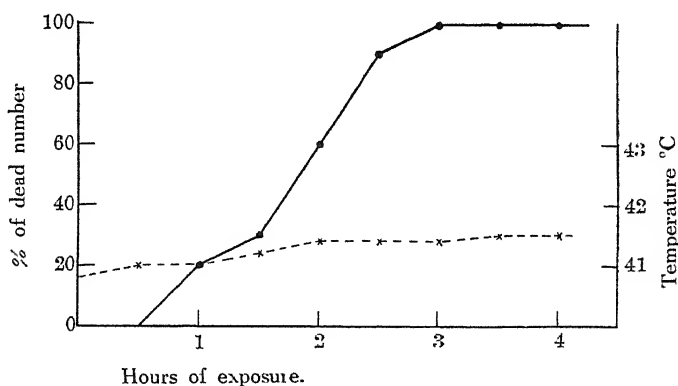


Fig 5 Duration of life of *Nerita japonica* on the sun-baked stone.

From these results, we can see that *Nerita japonica* could not live on the dry stone where the sun shines directly.

General Remarks in regard to Duration of Life. Despite of the fact that *Nerita japonica* under experimental conditions can live for a considerably longer time in tap water and diluted or concentrated sea water, though the individual variations are large, but in the field, these animals are found to be more common on the wet surface of rocks than in the water. It seems improbable that these animals ever remain in tap water in nature

beyond 3 days, the period within which the animals can survive, since before their habitats change to a water pool by heavy rain, they would soon creep away and seek a more suitable place. Similarly the dilution of salinity of a tide pool by the rain or, concentration by sun shine, may be avoided by creeping away long before the water becomes unsuitable for their life. They are always found only in the tide pool where the salinity was higher than 3.0 and lower than 4.0. I measured the salinity of the tide pool of various weather where the animals were living and found that the value of the salinity of such pools was 3.8-3.3. Therefore these values of salinity might be most suitable for their life.

The results of experiment II shows that *Nerita japonica* could live for more than one month in the air, and when directly exposed to the sun they could not live more than 3.5 hours on the dry rock. Therefore these animals avoid the dryness and direct sun shine concealing themselves in a crevice or hole in the rocks during dry-time. In nature, *Nerita japonica* are absent or rarely found on the sun-baked area above the high water neaps but are found either on the rocks sheltered from the sun during the greater part of the day or in places which are damp even on summer days. The intensity and duration of sun light appears to be the main factor in the limitation of their habitat.

TROPISMS

The examinations of tropism phenomena of the animals seem very important on the study of the animal behavior.

I Geotropism. If *Nerita japonica* are placed in a water tank, they creep up on the shaded vertical plane. This may be an effect of the gravity and the light. In the dark room, when the animals are placed on a frosted glass plate which is in water or in the air and inclined in various angles they creep upward immediately, whether they were placed head side down or not. Indeed the glass plate was inclined at 15, 30, 45, and 60 degrees respectively but in every case the animals crept straight forwardly and thus distinctly show the negative geotropism.

In the lighted room, when the animals were placed on the glass plates which were inclined less than 45 degrees, some of them crawled sideways, some upwards, and still others downwards. When however the inclination angles of the plates were increased, the majority of them show the negative geotropism.

II. Phototropism. When *Nerita japonica* are kept in a water tank,

they move along the vertical wall of the tank, but during the night the animals creep very actively and come out from the tank. Every morning I saw that almost all the animals which were placed in the tank on the previous evening came out from the tank and assembled in the shaded corners of the floor.

In the dark room, sometimes these animals creep straightly but sometimes irregularly as are shown in Figure 6. Thus the direction of their locomotions are not fixed, some rotate leftwards and others turn to the right.

In the lighted room, I found that all animals show negative phototropism, whether their heads faced the light direction or not. Figure 7 shows the results of the effect of sun light.

Then I examined their phototropism in the dark room by using an electric lamp. As the light source an electric lamp of 30 watts or 60 watts was used and 3 experiments were carried on under the influence of a horizontal light at the distance of 30, 50, and 100 centimeters respectively from the light source. In each experiment 30 animals were

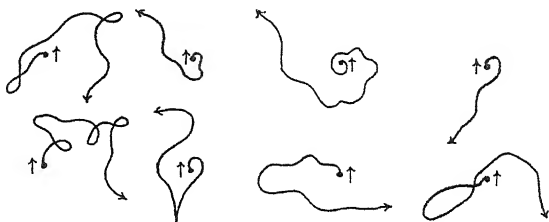


Fig. 6. Traces of movements of *Nerita japonica* in the dark room, small arrow indicates the direction of the head

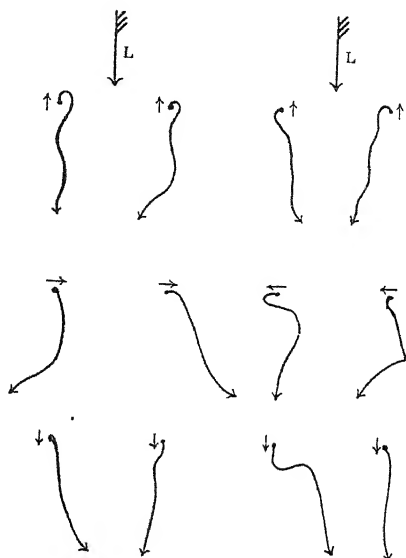


Fig. 7. Showing the effect of light on the movements of *Nerita japonica*. L, direction of light; small arrow indicates the direction of the head.

used. The animals were placed on the starting point so as to face their heads toward the light. The animals open their operculum gradually.

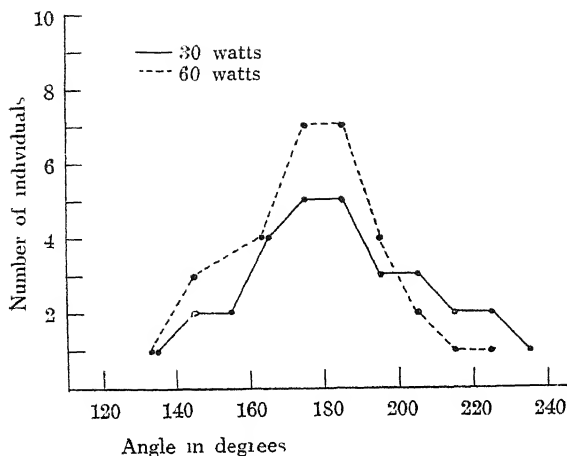


Fig. 8 Phototropism—Effects of an electric lamp. 30 cm from the light source.

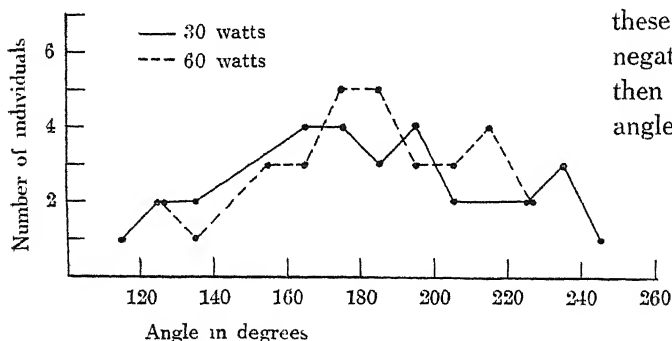


Fig. 9 Phototropism—Effects of an electric lamp 50 cm. from the light source.

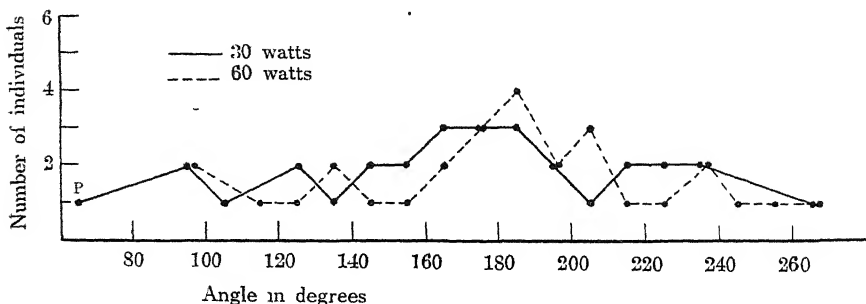


Fig. 10. Phototropism—Effects of an electric lamp. 100 cm. from the light source.

rotate and creep away from the light. The angles of their retreat to the line which passes the light source and the start point were measured counterclockwise, angle of 10 degrees is taken as unit. When the animal creeps away from the light straightly or vertically to the left, its angle with the line shows 180° or 90° respectively. The angles vary in every case, but if these animals show the negative phototropism, then the inclination angles of their retreat

must lie between 90° and 270° . The results of these experiments are shown in Figures 8, 9, and 10. Each figure shows a frequency curve which has its maximum at 180° and the value of its frequency lie between 90° and 270° excepting only one individual in Figure 10 marked P. This means that these animals have negative phototropism. The experimental results in the case of 60 watts are more distinct than that of the case of 30 watts in every case and, moreover, the greater the distance of the startpoint from the light, the larger in the increase of fluctuation of the angles of their retreat. That is, the distinctness of the animals' reactions to the light decreases in accordance with the increases of the distance from the light source or with the decreases of the intensity of the light.

III. Rheotaxis. Two methods used in this experiment, one is a glass tube (length 60 cm., diameter 2.5 cm.) and another is shown in Figure 11.

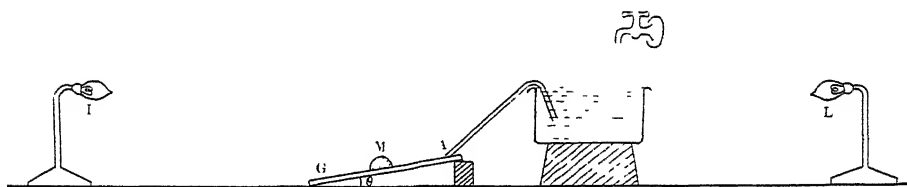


Fig. 11 Showing the method of investigation of rheotaxis. $AL=AL'=1$ meter
G, frosted glass plate, L & L', electric lamp; M, material, θ =inclination angle, 5 degrees

For the exception of the effect of light, I carried this experiment in the dark room.

The glass tube was placed horizontally on the floor and from one end was flowed water with a constant pressure. The volume of running water is at the rate of about 400 cc. per minute, through a glass tubing of 5 mm. In the tube only one individual was placed. The animal crawled very slowly and reached the source of the current, whether faced initially to the water source or not. The animal facing opposite the water source, turned in the tube and crawled towards the source.

In the second method, 5 animals were placed in a line with intervals of 3 cm. from one another and the positions were noted after 20 minutes. The volume of water is at the rate of about 170 cc. and 400 cc. per minute, through a glass tubing of 3 mm. and 5 mm. respectively (Table IX).

Whether the animals were tested immediately after taken from the water or after exposed to the air for more than 2 hours before being used, the results were the same.

TABLE IX.
Rheotaxis of Nerita japonica in the dark room.

Diameter of glass tube in mm	Angle of degrees	Number of animals indicating positive rheotaxis	Number of animals indicating negative rheotaxis	Number of no-moved animals	Number of sideway-moved animals
3	0	31	1	11	7
	180	28	3	14	5
5	0	37	2	1	7
	180	25	5	2	18

Angles 0° and 180° mean that the animals faced to and opposite to the current direction respectively.

From these experiments, I can conclude that the rheotaxis of *Nerita japonica* is positive.

Then I examined which factor acts more strongly upon the animals, when two factors, light and water current, are acting together at the same time. In figure 11, *L* is an electric lamp of 30 watts for the light source. When the sources of water and light were of the same direction, the animals did not advance towards the water source, as shown in Table X.

TABLE X.
Rheotaxis of Nerita japonica, acting together the light and water current at the same time from the same direction.

Diameter of glass tube in mm.	Angle in degrees	Minutes after	Numbers of animals indicating positive rheotaxis	Numbers of animals indicating negative rheotaxis	Numbers of no-moved animals	Numbers of sideway-moved animals
3	0	10	0	10	20	0
		20	0	14	15	1
		30	1	19	9	1
	180	10	0	25	5	0
		20	0	26	1	0
		30	0	28	2	0
5	0	10	0	8	19	3
		20	0	11	16	3
		30	0	12	15	3
	180	10	0	10	18	2
		20	0	15	9	6
		30	1	15	8	6

Angle 0° or 180° means that the animals faced to or opposite to the current direction respectively.

TABLE XI.

Rheotaxis of Nerita japonica, acting together the light and water current at the same time from the opposite direction.

Diameter of glass tube in mm	Angle of degrees	Minutes after	Numbers of animals indicating positive rheotaxis	Numbers of animals indicating negative rheotaxis	Numbers of no-moved animals	Numbers of sideway-moved animals
3	0	10	15	0	5	0
		20	17	0	3	0
		30	18	0	2	0
	180	10	12	0	7	1
		20	15	0	4	1
		30	16	0	3	1
5	0	10	14	0	6	0
		20	16	0	4	0
		30	18	0	2	0
	180	10	10	0	10	0
		20	14	0	6	0
		30	16	0	4	0

Angle 0° or 180° means that the animals faced to or opposite to the direction of current respectively

But on the contrary, when the directions of light and water current were the reverse (light source is *L'* in Fig. 11), all animals reached the source of the current (Table XI). These facts indicate that the phototropism is more important than the rheotaxis for the determination of its habitat.

SUMMARY

1. The habitat of *Nerita japonica* DUNKER is on the cliffs which are sheltered from the sun during the greater part of the day. They live in crevices or in the holes of rocks at about high-water neaps.

2. *Nerita japonica* is found on the rock but very rarely on the sand. They can also creep on the wet sand.

3. The animals move actively on rocks not only wetted by the waves, but also by the rain.

4. The animals can do forward locomotion but not backward locomotion.

5. *Nerita japonica* moves about 2 meters for a day along the slit of the rock and stopped at about the high water mark of the neaps.

6. The animals have no definite home.

7. *Nerita japonica* can live for a considerably longer time in variously changed water but on the dry rock and under direct sunlight they can survive only 3.5 hours long. The dryness appears to be the main limiting factors to the determination of their habitat.

8. *Nerita japonica* exhibits negative geotropism, negative phototropism and positive rheotaxis, but on the movement of the animals the light acts more strongly than the water current.

REFERENCE

- ABE, N. 1931. Ecological Observations of *Acmaea dorsuosa* GOULD. Sci Rep Tōhoku Imp Univ, Biol., Vol. VI, No. 3
- AINSWORTH DAVIS, J. R. and FLEURE, H. J. 1903. *Patella* (The Common Limpet). L. M. B. C. Memories X, London
- MITSUKURI, K. 1901. Negative Phototaxis and other Properties of *Littorina* as Factors in Determining its Habitat. Annot Zool. Japon, Vol. IV
- NOMURA, E. 1916. Effect of Light on the Movements of Earthworm, *Allolobophora foetida* (SAV.). Sci Rep Tōhoku Imp. Univ, Biol, Vol. I, No. 4
- ORTON, J. H. 1928. Observation on *Patella vulgata*. Jour. Mar. Biol. Associ., Vol. XV (NS)
- PARKER, G. H. 1911. The Mechanism of Locomotion in Gastropods. Jour. Morph., Vol. XXII, No. 1.

SOME NOTES ON *MUSCULIUM HETERODON* (PILSBRY), A FRESHWATER BIVALVE

I. THE GENITAL SYSTEM AND THE GAMETOGENESIS

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(With 9 figures in text)

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As is widely known, the molluscan family Sphaeriidae is represented by one of the three genera, *Sphaerium*, *Musculium*, and *Pisidium*. Many European and American investigators have described the anatomy, embryology, and life history of the several species of the genus *Sphaerium*. In *Cyclas cornea* (*Sphaerium corneum*), LEYDIG (1855) first investigated the general anatomy, and then ZIEGLER (1885), STAUFFACHER (1894), and MEISENHEIMER (1901) were concerned with the embryonic development or organogeny. Recently MONK (1928) studied the general anatomy of *Sphaerium notatum*, and WOODS (1931, '32) described the history of the germ cells, and reported the so-called "Keimbahn determinant" in relation to *Sphaerium striatinum*.

While all these papers are concerned with several species of *Sphaerium*, hitherto no reports have been published with regard to the Japanese species of *Musculium*. In the belief that some valuable informations may be derived from the embryological study of the Japanese material, the present work was undertaken in April, 1933, at the suggestion of Prof. Dr. E. NOMURA

The writer is indebted to Prof. E. NOMURA, under whose direction and guidance the study has been conducted throughout the progress of the work. My thanks are also due to Assist.-Prof. I. MOTOMURA for his advice.

MATERIAL AND METHOD

The material used in the present study is *Musculium heterodon* (PILSBRY), which belongs to the subfamily Sphaeriinae of the family Sphaeriidae (BAKER 1927).

The species under discussion is one of small eulamellibranchs, the

specimens measuring 9.5 mm. in length and 5.8 mm. in height even in the largest hitherto obtained (NOMURA 1926).

The embryonic shell is retained distinctly at the peculiarly bulged umbo (Fig. 1). The outer surface of the shell is yellowish gray, lighter at the margins, and is accurately marked with a series of fine growth lines, some of which are prominent and responsible to so-called "lines of annual growth."



Fig. 1 Left side view of two specimens of *Musculium heterodon*, to show general contour, especially embryonic shell at umbo. Photo $\times 2$. When the shell is dried up, the embryonic shell is easily observable

The specimens were collected from a drainage-ditch in Sendai, on the 15th day of every month.

In order to avoid the muscular contraction, they were first put into hot water, which was heated gradually from 35°C. to 70°C., and then, after becoming insensitive, they were killed in a fixing solution. Zenker's solution containing 3% of glacial acetic acid was satisfactory for the histological study, and Allen-Bouin's for the cytological study. After the fixation, the shells were removed from the soft part of the specimens by a pair of fine forceps. Then, in order to decalcify the young mussels remaining within the gill, 70% of alcohol containing 1% of acetic acid was used for two or three days. Finally the completely decalcified material was preserved in 90% of alcohol.

In carrying out all the present investigations, the paraffin serial sections measuring 5–8 μ were used for the cytological and histological observations, and those measuring 10–20 μ for the anatomical reconstruction.

Of several methods of staining tried, the combined stain of Heidenhain's hematoxylin and orange G, or Mallory's connective tissue stain, was satisfactory.

GENITAL SYSTEM

In the species under investigation, as in the other lamellibranchs, the visceral sac involves the organs of several systems. The genital system

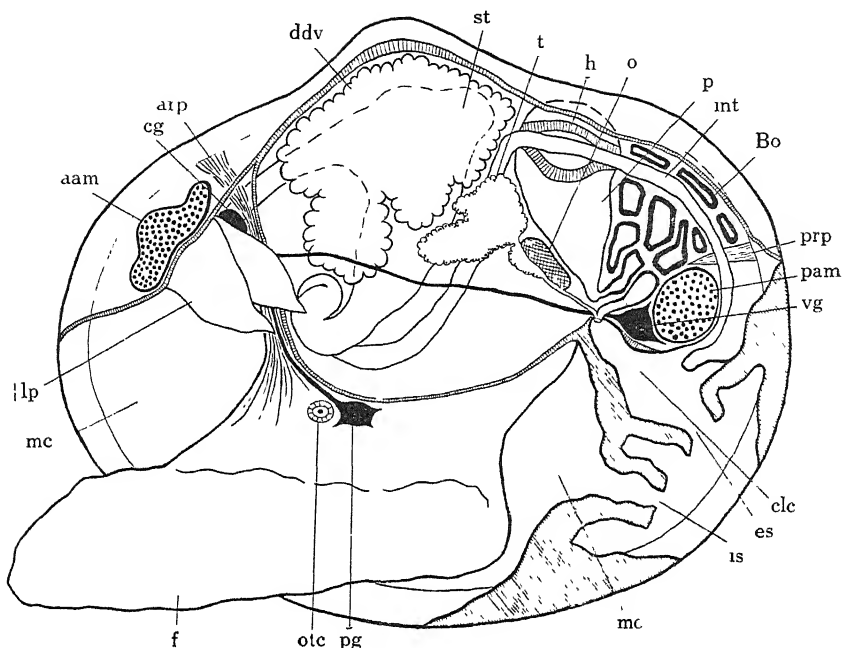


Fig. 2 Schematic representation of inner organization of *Musculum heterodon*, viewed from left side. Reconstructed. Mantle, gills, and several walls on left side of body removed; section edges hatched with parallel lines. aam anterior adductor muscle, arp anterior retractor pedis, Bo Bojanus' organ, cg cerebral ganglion, clc cloacal chamber, ddv digestive diverticula, es exhalant siphon, f foot, h heart, int intestine, is inhalant siphon, lp labial palpi, mc mantle cavity, o ovary, otc otocyst, p pericardium, pam posterior adductor muscle, pg pedal ganglion, prp posterior retractor pedis, st stomach, t testis, vg visceral ganglion

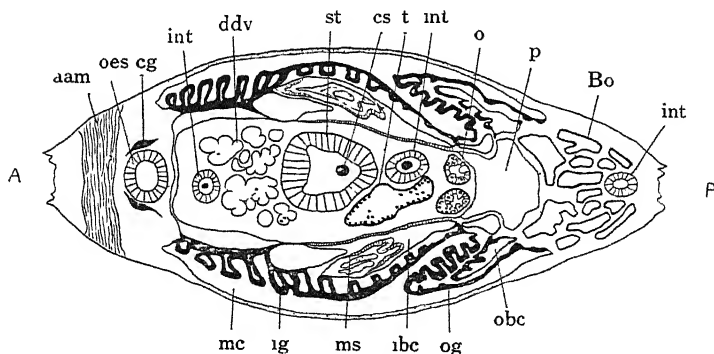


Fig. 3. Schematized horizontal section through gonads and anterior adductor muscle, to illustrate topographical relation between visceral organs. A anterior, P posterior, aam anterior adductor muscle, Bo Bojanus' organ, cg cerebral ganglion, cs crystalline style, ddv digestive diverticula, lbc inner branchial chamber, ig inner gill, int intestine, mc mantle cavity, ms marsupial sac, o ovary, oes oesophagus, obc outer branchial chamber, og outer gill, p pericardium, st stomach, t testis.

is found in the loose connective tissue of the visceral sac, and extends from the anterior portion of the pericardium posteriorly to Bojanus' organ (Figs. 2 and 3).

The mature gonad is not found until the animal attains to a length of about 2.5 mm. The genital system of the sexually mature animal consists of one irregularly lobed testis with paired sperm ducts, a pair of tubular ovaries, and a pair of hermaphroditic ducts (Fig. 1).

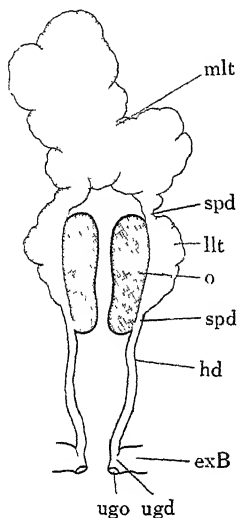


Fig. 4. Schematic representation of genital system. Reconstructed. *exB* external duct of Bojanus' organ, *hd* hermaphroditic duct, *llt* lateral lobe of testis, *mlt* median lobe of testis, *o* ovary, *spd* sperm duct, *ugd* urinogenital duct, *ugo* urinogenital orifice.

On the respective side of the body, each hermaphroditic duct runs into the external duct of Bojanus' organ, and forms there, together with the latter, the short urinogenital duct, which opens into the cloacal chamber through the urinogenital orifice. Histologically, the structure of the wall of the urinogenital duct and orifice resembles that of the external end of the renal organ more than that of the hermaphroditic duct.

Anteriorly to the urinogenital duct, the hermaphroditic duct proceeds along the antero-ventral wall of the pericardium, and divides into two branches, viz. inner the oviduct, and outer the sperm duct, at the level of half the height of the pericardium. In large specimens, the length of the hermaphroditic duct is about

0.5 mm. with a diameter of $50\ \mu$. Its wall consists of $10\ \mu$ -cuboidal cells which form a non-ciliated, unicellular layer. At the upper termination of the hermaphroditic duct the ovary forms a tubular blind sac, the oviduct being thus so short that it may be stated that the hermaphroditic duct opens directly into the ovary.

In the mature ovary (Fig. 5), the outermost layer is composed of connective tissue cells compactly arranged, and forms the basement membrane of the ovarian epithelium. The ovarian epithelium is a unicellular layer, adhering outside to the basement membrane and, inside, facing towards the ovarian cavity, which is the central space of the ovary. It is composed of tall columnar cells, and contains ova in several stages of

growth which are interposed between the epithelial cells, or intercalated between this layer and the outer basement membrane.

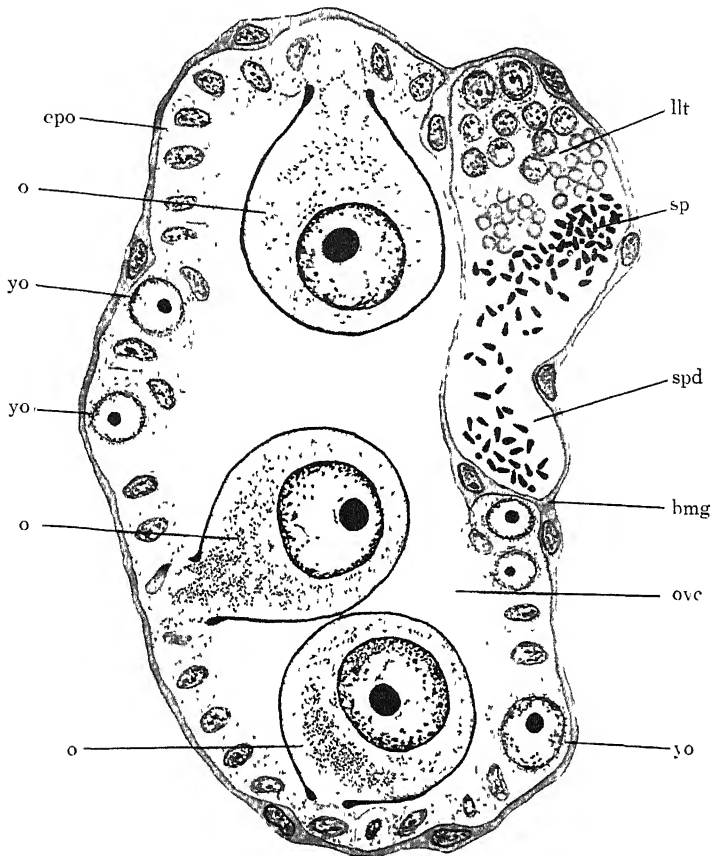


Fig 5 Section of gonads, to show relation between testis, sperm duct, and ovary $\times 700$ *bmg* basement membrane of gonads, *epo* ovarian epithelium, *llt* lateral lobe of testis, *o* ovum, *ovc* ovarian cavity, *sp* spermatozoa, *spd* sperm duct, *yo* young ovum

The sperm duct continues to the uppermost end of the hermaphroditic duct, from which the ovary branches out, and proceeds anteriorly to the testis, being closely attached to the outer surface of the ovary. The adult testis is divided into three lobes, a median and paired lateral, each of which is irregularly folded (Fig. 4). The median lobe is massive, and is the main portion of the testis, forming the most anterior portion of the genital system. This portion of the testis extends to the loose connective

tissue near the digestive diverticula, and is dislocated towards the left side of the body, the corresponding right side being occupied by the alimentary canal (Fig. 3). Each lateral lobe is smaller and more slender than the median lobe, and lies just posterior to the latter, a little in front of the level of the ovary.

The mature testis is a thick walled sac with a central cavity irregularly formed, its outer surface being covered with the basement membrane (Fig. 6).



Fig. 6. Section of mature testis, to show its general structure. Photo. $\times 230$.
ibc inner branchial chamber, *int* intestine, *lcc* loosely scattered connective tissue cells, *mlt* median lobe of testis, *owv* outermost wall of visceral sac

The sperm duct opens at first in the cavity of the lateral lobe of the testis and then extends anteriorly to that of the median lobe. Its wall is continuous with the basement membrane of the ovary and testis, and I was not able to find any epithelial structure attached to it.

In my opinion, the whole genital system ought to be bi-laterally symmetrical in its constitution, but, as the result of the dislocation stated above, in the adult testis a single median lobe has been formed, this formation being probably due to a union of the paired groups of the primordial spermatogonia.

The median lobe of the testis is well developed in smaller specimens, but in larger ones the period of sperm formation in the lobe has already passed, while the spermatogenesis is still active in the lateral lobes. Thus it seems to me that the maturation in the testis proceeds from the anterior end to the posterior.

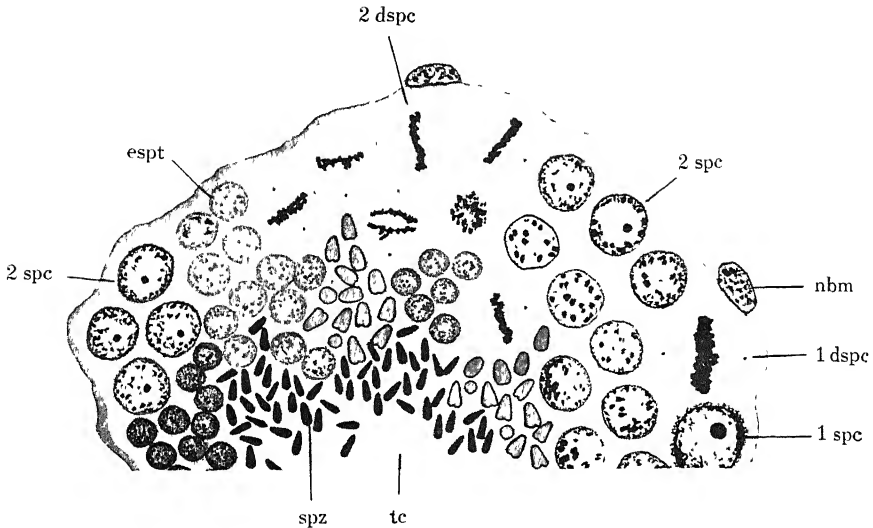


Fig 7 Section of testis, to illustrate different stages of spermatogenesis. $\times 1000$. *1 dspe* first division of spermatocyte, *2 dspe* second division of spermatocyte, *1 spc* primary spermatocyte, *2 spc* secondary spermatocyte, *espt* early stage of spermatid, *nbm* nucleus of basement membrane, *spz* spermatozoa, *tc* cavity of testis.

In the wall of the testis, spermatogonia and spermatocytes are located peripherally, but numerous spermatids and spermatozoa are found always next to the cavity (Fig. 7), thus revealing that the maturation process in a given lobe of the testis progresses from the center towards the periphery, and, therefore, the stages of spermatogenesis are easily traceable.

SPERMATOGENESIS

In the mature testis the spermatozoa are found throughout the year, though their number is less in the winter season than in the others. In my material as prepared, the outline of the chromosomes was always so hazy and their number was so indeterminable, that it was not suitable for the study of cytological details. Yet the spermatogenetic stages could be distinguished, and it may be stated that during the metaphase or anaphase in the meiosis all the chromosomes are of about the same size and shape (Fig. 8).

WOODS (1931) states in his study of *Sphaerium striatinum* that four of the chromosomes in the second spermatocyte are always in advance of the others as they pass towards the poles. Somewhat similar cases (Fig. 8 K) were also met with in my species.

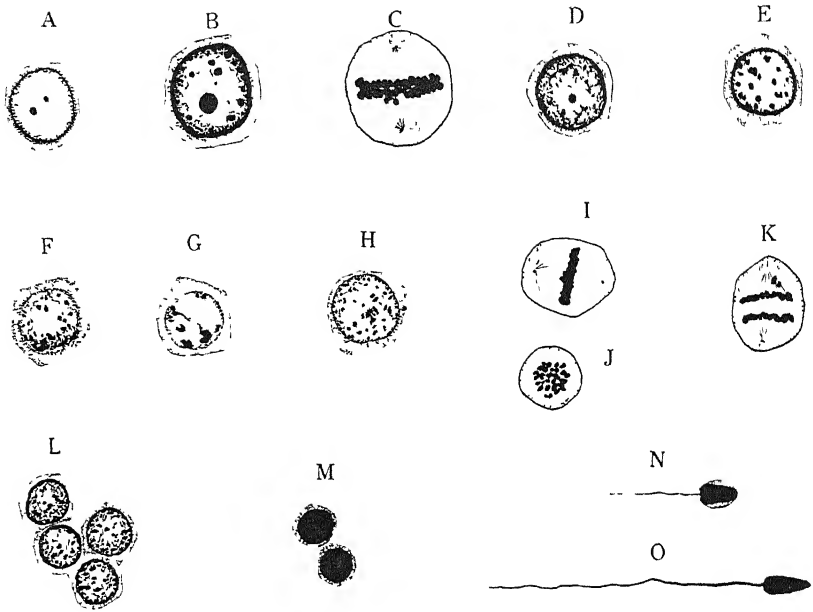


Fig 8 Stages of spermatogenesis $\times 1400$

- A spermatogonium
- B primary spermatocyte
- C metaphase of first division of spermatocyte
- D secondary spermatocyte
- E, F, G and H prophase of second division of spermatocyte.
- I and J metaphase of second division of spermatocyte.
- K anaphase of second division of spermatocyte.
- L spermatid
- M and N spermatids in spermiotelsons.
- O complete spermatozoon

SPERMATOZOON

The head of the complete spermatozoon of the present species is about 5μ in length, and its frontal view shows an elongated fusiform shape, somewhat rounded posteriorly (Fig. 8 O). The middle piece could hardly be observed in the present spermatozoon, and the lack of the middle piece may perhaps be a common characteristic in some molluscan species, such as *Modiolaria marmorata* and *Cardium exiguum* after LOVÉN (1848), *Ostrea virginiana* after BROOKS (1880), and *Limax agrestis* after BYRNES (1899).

According to MONK (1928), in *Sphaerium notatum* the spermatozoon is fusiform and is destitute of tail. In my material, the tail is easily observable.

GROWTH OF THE OOGONIUM

Various developmental stages of the oogonia, ten or more in a simultaneous stage, are found in the mature ovary throughout the year. The

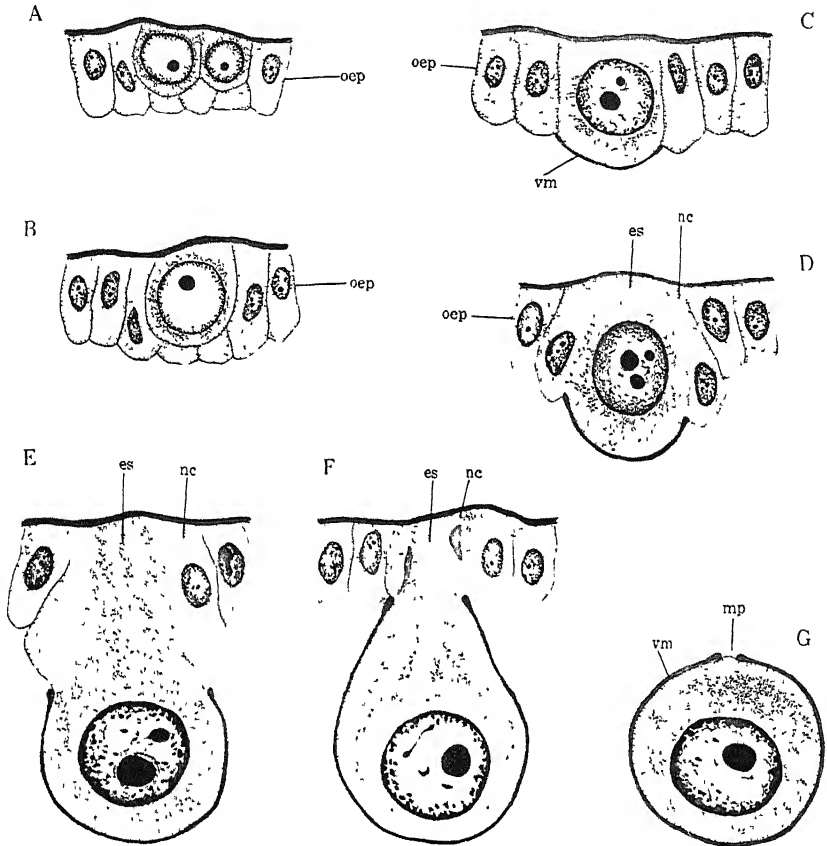


Fig. 9. Stages of oogenesis $\times 700$ *es* egg stalk, *mp* micropyle, *nc* nurse cell, *oep* ovarian epithelium, *vm* vitelline membrane

A and *B* oogonia. early stage still in basal half of ovarian epithelium

C same: distal surface projected to ovarian cavity; covered with egg membrane.

D same: more protruded towards ovarian cavity, egg membrane more spreading over its free distal surface, egg stalk forming at its proximal half; nurse cells differentiating from ovarian epithelial cells around it

E same: much more protruded into ovarian cavity; egg membrane much more spreading; egg stalk and nurse cells highly enlarged; oogonial nucleus being dislocated towards distal half.

F same: its protrusion and formation of egg membrane almost completed; egg stalk and nurse cells showing tendency to degeneration

G primary oocyte

oögonia in the early stages are found intercalated between the ovarian epithelium and its basement membrane (Fig. 9 *A* and *B*). Each oögonium is spherical or oval in shape and is less tall than the neighbouring epithelial cells, which are columnar. Each oogonial nucleus is spherical and vesicular, almost filling up the oögonium. It is much larger than those of the epithelial cells, which are always ovoidal or ellipsoidal, with their longest axis perpendicular to the basement membrane, and contains, invariably, a plasmosome distinctly larger than those in the epithelial cells, and a small amount of fine granular chromatins distributed peripherally.

Each oögonium grows and pushes its way between the epithelial cells and begins to protrude towards the ovarian cavity (Fig. 9 *B*).

As soon as the distal surface of the oögonium has reached the ovarian cavity, the actual portion just exposed to the cavity becomes covered by an egg membrane (Fig. 9 *C*). In the nucleus, the granular chromatins increase in number and one or two accessory plasmosomes appear. The cytoplasm becomes rich in nutritive substance, and stains strongly on the applying Heidenhain's hematoxylin.

In the following stage, the distal portion of the oögonium continues to protrude more into the ovarian cavity, while the proximal portion narrows and forms the egg stalk, being oppressed by the adjacent epithelial cells (Fig. 9 *D*). The egg plasm, as a whole, is in the act of flowing from the proximal portion to the distal. The nucleus increases in size immensely and is dislocated from the original position to the newly-protruded, distal portion of the oögonium. The chromatin granules begin to be rearranged into threads forming a fine network from an original, diffusely distributed condition. Besides one large plasmosome, two or three small plasmosomes become visible. The nurse cells — some ovarian epithelial cells adjacent to the oögonium — tend to be elongated while the oögonium continues to be enlarged.

In the later stages of growth, the distal portion of the oögonium, still enlarging, continues to project into the ovarian cavity, and the whole projected portion is covered by the egg membrane, which shows a slight but distinct elevation around its proximal end (Fig. 9 *E*). The proximal egg stalk still continues to narrow. The boundary between the egg stalk and the nurse cells is not so indistinguishable, but no cell membrane is visible there. In the portion proximal to the nucleus, fine granules are specially aggregated. Woods (1932) has also observed similar granules in *Sphaerium striatinum*, and identifies them with the mitochondria. In the nucleus, the chromatins become again granular and there appear

usually two plasmosomes, a larger and a smaller. The nurse cells are at a maximum elongation.

In the last stage of growth, the oögonium is suspended only by its egg stalk in the ovarian cavity from the ovarian wall. The nucleus becomes much enlarged and vesicular, containing only one large plasmosome. The fine so-called mitochondria become a little coarser. Occasionally, I was able to find two special granules closely arranged, just outside the nucleus at its proximal side. They take a plasma-stain and are nothing but the centrosomes. STAUFFACHER (1894) has already found centrosomes in *Sphaerium corneum*. The elongated nurse cells withdraw again to the level of the original epithelium, and show several signs of degeneration. By this time, the egg membrane is almost completed (Fig. 9 F).

Finally the liberation of the oögonium into the ovarian cavity occurs at the close of the growth period, the degenerating nurse cells and the egg stalk outside the egg membrane being remained in the epithelium. As to the fate of the nurse cells, I have nothing to report at present, but it appears to me that they continue further degeneration and never regain their function.

REMARKS ON THE GROWTH OF THE OÖGONIUM

1) THE VITELLINE MEMBRANE. A thick and clear egg membrane is reported in most of freshwater lamellibranchs (*Unio*, *Anodonta*, and *Sphaerium*). In *Musculium heterodon*, the egg membrane is formed as soon as the distal surface of the oögonium reaches the ovarian cavity, and there is no gland which may secrete any egg membrane (Fig. 9 C). It may therefore be true to say that the contact of the naked egg plasm with the fluid contained in the ovarian cavity produces a piece of the membrane. The latter then spreads continuously to a primary envelope, while the protrusion of the oögonium and the withdrawing of the nurse cells continue. Thus, in my opinion, the egg membrane of the present species is no doubt the vitelline membrane, as is defined by several authorities in other species.

2) THE ACCESSORY PLASMOSOMES. Not only in Sphaeriidae but also in many other molluscs, two or three plasmosomes have been reported by many authorities during the period of growth. According to STAUFFACHER (1894) and others, the presence of accessory nucleoli is due to budding from the main nucleolus. In my case, the appearance of accessory small plasmosomes is in the middle period of growth (Fig. 9 C and D), but I

was not able to find them in the early and later periods. In reality, the formation of the accessory plasmosomes happen to occur with the increase of the amount of nuclear contents, granular and fibrillar. At first a few small plasmosomes appear here and there in the nucleus, besides the main plasmosome which is always perceptible throughout the whole period of growth. But, later, the accessory plasmosomes fuse with the main plasmosome, a single large nucleolus resulting. In my opinion, the appearance of the accessory plasmosomes is thus a transitional phenomenon in the formation of a single larger plasmosome by the addition of smaller ones. The process of fusion is repeated once or twice, and, finally, the main plasmosome becomes the germinal spot in the primary oöcyte.

3) THE NURSE CELLS. Some ovarian epithelial cells, adjacent to each oögonium, have now been named by myself the nurse cells. While the oögonium continues to grow, these nurse cells are in most intimate contact with it. They increase in bulk and are elongated along the egg stalk. The boundary between the nurse cells and the oögonium becomes obscure, and the egg membrane is never formed there, notwithstanding its presence covering the whole free surface of the oögonium. Moreover, in the stages of completion of the growing period, the nurse cells withdraw, showing a tendency to degeneration, to the level of the original epithelium (Fig. 9 *F*). Such facts may suggest to us the possibility that the nurse cells supply nourishment to the oögonium during its growth, as in the case of the insect eggs reported by KORSCHOLT (1884).

PRIMARY OÖCYTE

At the end of the growth stage, each full-grown oögonium is liberated from the ovarian epithelium into the ovarian cavity, and becomes the primary oöcyte. The primary oöcyte is almost spherical with a typical germinal vesicle containing a germinal spot. Its diameter measures about $40\ \mu$. The germinal vesicle is somewhat eccentric in situation, and is somewhat flattened, measuring about $15\ \mu$ in height and about $20\ \mu$ in breadth.

The vitelline membrane is quite distinct. It is perforated at the point of its attachment to the ovarian epithelium, and forms the micropyle. The margin of the vitelline membrane around the micropyle is a little elevated.

The original distal point of the oöcyte opposite the micropyle corresponds to the animal pole, from which the polar body will be formed

later. But, actually, the formation of the polar body never occurs while the oöcytes are in the ovarian cavity. Perhaps the maturation division will occur after liberation of the oöcytes to the cloacal chamber.

The egg plasm is homogeneous containing a small amount of deutoplasm, but, in the vegetal portion, dark particles of mitochondria are scattered somewhat densely (Fig 9 G).

SUMMARY

1) *Musculium heterodon* is hermaphroditic; the genital system consists of a massive testis, and a pair of sperm ducts, tubular ovaries, and of hermaphroditic ducts.

2) The spermatozoa are found almost throughout the year; head elongate fusiform, 5μ in length, circular in cross section with a diameter of about 1.5μ in its widest portion; tail slender; destitute of the middle piece.

3) The oögenesis appears periodically, but throughout the year.

4) The oögonia of early stages are found intercalated between the ovarian epithelium and its basement membrane; they grow and protrude towards the ovarian cavity and finally develop into the oöcytes.

5) By this process of growth, formation of the vitelline membrane, dislocation of the egg nucleus, appearance of the accessory plasmosomes and their fusion into the main plasmosome, and differentiation and degeneration of the nurse cells happen to occur.

6) The primary oöcyte is homolecithal, nearly spherical, about 40μ in diameter.

REFERENCES

- AWATI, P. R. and RAL, H. S. 1931 *Ostrea cucullata*. Indian Zoological Memoirs Lucknow.
- BAKER, F. C. 1927. On the Division of the Sphaeridae into two Subfamilies; and the Description of a New Genus of Unionidae, with Descriptions of New Varieties. Amer. Mid. Nat., 10.
- BROOKS, W. K. 1880. Development of the American Oyster, (*Ostrea virginiana* LIST.). Rep. Comm. Fish. Maryland, 4.
- BYRNES, E. F. 1899 The Maturation and Fertilization of the Egg of *Limax agrestis* (LINNÉ). Jour. Morph., 16.
- COE, W. R. 1932. Sexual Phases in the American Oyster (*Ostrea virginica*). Biol. Bull., 63.
- COKER, R. E. 1922. Natural History and Propagation of Freshwater Mussels. Bull. U. S. Bureau of Fish., 37.
- GATENBY, J. B. 1917. The Cytoplasmic Inclusions of the Germ-Cells. Part I Lepidoptera. Quart. Jour. Micro. Sci., 62.

- GATENBY, J. B. 1917 The Cytoplasmic Inclusions of the Germ-Cells. Part II. *Helix aspersa*. Ibid., 62
- GILMORE, R. J. 1917. Notes on Growth and Reproduction in Certain Viviparous Molluscs of the Family Sphaeriidae Nautilus, 31.
- HARMS, W. 1908. Die postembryonale Entwicklung von *Unio pictorum* und *Unio tumidus*. Zool. Anz., 32
- JHLRING, H. von 1877 Zur Kenntniss der Eibildung bei den Muscheln. Zeits. wiss. Zool., 29
- JOHNSTONE, J. 1899. *Cardium*. L. M. B. C. Memoirs. Liverpool
- KORSCHULT, E. 1884 Über die Bildung des Chorions und der Mikropylen bei den Insekten-eiern Zool. Anz., 7.
- LEFEVRE, C. and CURTIS, W. C. 1910. Reproduction and Parasitism in the Unionidae. Jour. exp. Zool., 9.
- LOVÉN, S. 1848 Beiträge zur Kenntniss der Entwicklung der Mollusca Acephala Lamelli-branchiata. K. Vet. Akad. Handl., Stockholm.
- MEISENHEIMER, J. 1901 Die Entwicklung von Herz, Perikard, Niere, und Genitalzellen bei *Cyclas* im Verhältniss zu den übrigen Mollusken Zeits. wiss. Zool., 69
- MONK, C. R. 1928. The Anatomy and Life-history of a Freshwater Mollusc of the Genus *Sphaerium*. Jour. Morph. Physiol., 45
- NOMURA, E. 1926. On Application of $a=kb'$ in Expressing the Growth Relation in the Freshwater Bivalve, *Sphaerium heterodon* PILS. Sci. Repts. Tôhoku Imp. Univ., Biol., 2
- ORTON, J. H. 1927 Observations and Experiments on Sex-Change in the European Oyster (*O. edulis*). Part 1. The Change from Female to Male Jour. Mar. Biol. Assoc. U. Kingd., 14
- ORTON, J. H. 1931. Observations and Experiments on Sex-Change in the European Oyster (*O. edulis*). Part 2. On the Gonad of Egg spawning Individuals Ibid., 17.
- STAUFFACHER, H. 1894 Eibildung und Furchung bei *Cyclas cornea* LAM. Jen. Zeits. Naturwiss., 28.
- STERKI, V. 1922. Some Notes on Sphaeriidae with Descriptions of New Species Ann. Carnegie Museum, 13.
- WOODS, F. H. 1931 History of the Germ Cells in *Sphaerium striatinum* (LAM.) Jour. Morph. Physiol., 51.
- WOODS, F. H. 1932 Keimbahn Determinants and Continuity of the Germ Cells in *Sphaerium striatinum* (LAM.) Ibid., 53

ZUR KENNTNIS DER ENTWICKLUNGSGESCHICHTE VON *HETEROCHORDARIA*, *SCYTOSIPHON* UND *SOROCARPUS*¹⁾

VON

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(Mit Tafel X und 6 Textfiguren)

(Eingegangen am 10. Oktober 1934)

Die hier vorläufig veröffentlichten Angaben beruhen hauptsächlich auf vom Winter 1933 bis zum Frühling 1934 in der biologischen Station zu Asamushi ausgeführten Versuchen. Als Material gebrauchte ich *Heterochordaria abietina*, *Scytosiphon lomentarius* und *Sorocarpus uvaeformis*; alle drei wuchern üppig in dieser Gegend.

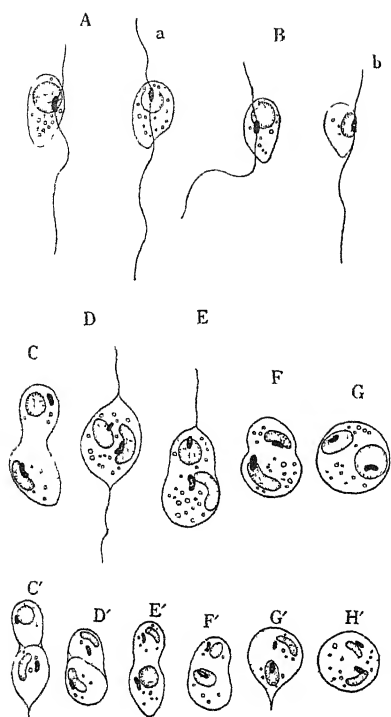
Wenn man reifes, vorher mit frischem Süßwasser oder filtriertem Meereswasser gründlich ausgespültes Material einige Stunden lang im Dunkeln stehen lässt und dann in ein Glasgefäß mit Meereswasser bringt, so bekommt man leicht eine grosse Menge von Schwärmern dieser Algen. Die Schwärmer zeigen starke negative Phototaxis.

Die Objektträgerkulturen wurden in Glasbechern mit etwa 200 ccm Wasser vor das Nordfenster eines ungeheizten Zimmers gestellt. Als Nährlösung benutzte ich SCHREIBERSche Flüssigkeit (1932).

I. *HETEROCHORDARIA ABIETINA* (RUPR.) S. AND G.

Heterochordaria abietina, eine Spezies der Heterochordariaceen, reift in Asamushi vom Winter bis zum Frühling. Bei dieser Alge findet man uni- sowie plurilokuläre Sporangien in getrennten Individuen. Die in unilokulären Sporangien entstandenen Schwärmer keimen gewöhnlich ohne Kopulation. Unter den Schwärmern gibt es aber zwei Arten (Textfigg. 1, A, a), welche verschieden gross sind und in getrennten, im Aussehen etwas verschiedenen Individuen (im Zeichen A, a) (Taf. X, Figg. 1, A, a), entstehen. Kopulation zwischen diesen zwei Arten von Schwärmern erfolgt auch recht häufig. 1928 veröffentlichte IKARI eine Mitteilung über die Entwicklung dieser Schwärmer, deren Kopulation er aber nicht beo-

¹⁾ Contribution from the Marine Biological Station, Asamushi, Aomori-Ken, No. 117.



Textfig. 1. *Heterochordaria abietina*. Viererlei Schwärmer und ihre Kopulation A-a Schwärmer aus unilokulären Sporangien von Individuen A und a, B-b Schwärmer aus plurilokulären von B und b C-G aufeinanderfolgende Stadien der Kopulation zwischen A und a, C'-H' dieselbe zwischen B und b Vergr. 1040

bachten konnte Nach meiner Erfahrung erfolgt die Kopulation nur in der lebhaftesten Zeit der Schwärmer.

Die Schwärmer aus unilokulären Sporangien keimen, wie allgemein bekannt, bei den meisten Algen nur ungeschlechtlich. Aber die Keimung nach der Kopulation solcher Schwärmer ist jüngst auch bei verschiedenen Algen beschrieben worden, z. B. bei *Pylaiella littoralis* (KNIGHT, 1923), bei *Ectocarpus siliculosus* (KNIGHT, 1929) und bei *Sphacelaria bipinnata* (CLINT, 1927). Also bietet der Fall bei *Heterochordaria abietina* nichts besonders Auffallendes.

Die in plurilokulären Sporangien entstandenen Schwärmer sind im Grunde Gameten, keimen aber auch ohne Kopulation. Bei diesen Schwärmern kann man auch zwei Arten unterscheiden (Textfigg. 1, B, b), die verschieden gross sind und in getrennten, verschieden aussehenden Individuen (im Zeichen B, b) (Taf. X, Figg. 1, B, b), entstehen.

Die Messung der Schwärmer ergab folgende Werte :

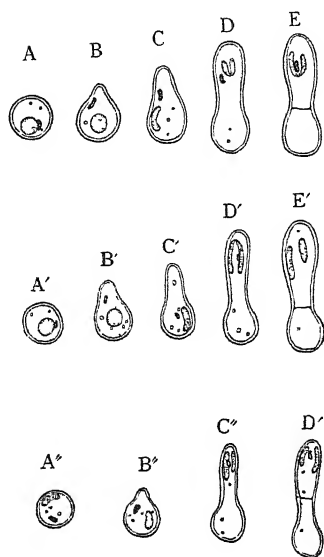
	Schwärmer aus A	aus a	aus B	aus b
lang :	9.3 μ	8.5 μ	8.2 μ	6.4 μ
breit :	4.7 μ	5.0 μ	4.5 μ	4.2 μ
abgerundet :	8.4 μ	8.0 μ	7.1 μ	6.0 μ

Sie alle besitzen je ein plattenförmiges Chromatophor und einen roten Augenfleck; der Augenfleck der kleineren Schwärmer ist etwas dunkler als der der grösseren. Mit Ausnahme der Schwärmer aus b beginnen diese Schwärmer nach etwa 24 bis 30 Stunden ungeschlechtlich zu keimen und entwickeln einen Keimschlauch, der auf der Unterlage hinkriecht.

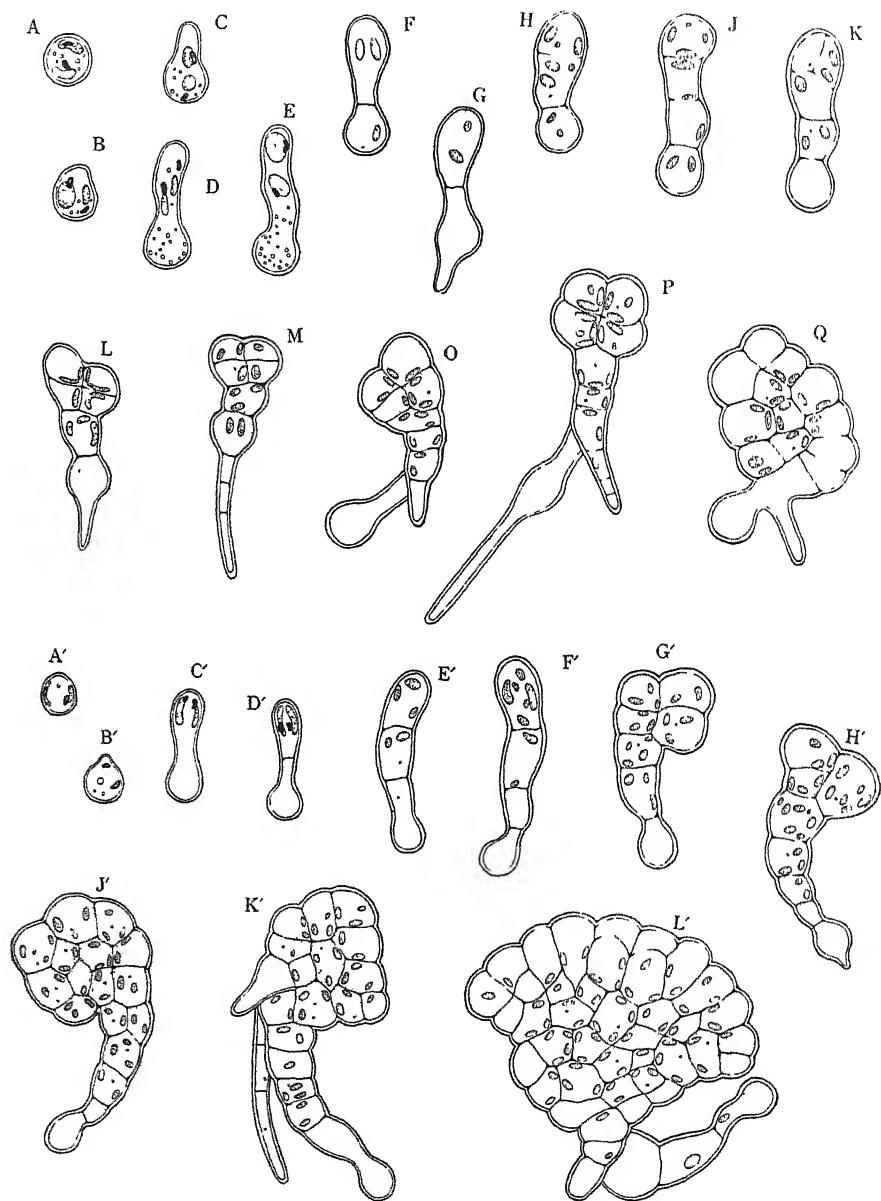
Die erste Querwand bildet sich nach etwa zwei oder drei Tagen (Textfig. 2). Bei der Keimung wandert der Inhalt der Schwärmer in den Keimschlauch hinüber, so dass nach der ersten Teilung des Keimlings die an der Spitze des Keimschlauchs abgeschiedene neue Zelle sehr inhaltsreich, die ursprüngliche Spore dagegen ganz leer wird. Durch eine Menge von Quer- und Längsteilungen beginnt sich dann die Scheibe zu bilden. Mit fortschreitender Entwicklung werden kriechende Wurzeln ausgebildet. Die vollständige Entwicklung zu neuen *Heterochordaria*-Individuen habe ich bei meinen Kulturen noch nicht verfolgen können.

Während die kleineren, aus *a* und *b* stammenden Schwärmer geraume Zeit beweglich sind, nehmen die grösseren aus *A* und *B* in kurzer Zeit eine runde Form an. Also verhalten sich diese weiblich und jene männlich. Beim Beginn der Kopulation schwärmen die männlichen massenhaft um die weiblichen. Die Textfigg. 1, *C-G*, *C'-H'* zeigen die aufeinanderfolgenden Stadien der Kopulation. Die Verschmelzung

erfolgt meistens derart, dass das farblose Ende des männlichen Schwärmers auf das das Chromatophor führende Ende des weiblichen trifft, ebenso wie bei *Ectocarpus siliculosus* (OLTMANN, 1922). Der Sexualakt selbst dauert 10 bis 15 Sekunden. Die Zygote umgibt sich bald mit einer Membran. Die beiden Chromatophoren in der Zygote liegen getrennt, ohne Verschmelzung. Der Durchmesser der Zygote der in unilokulären Sporangien entstandenen Schwärmer beträgt etwa 9.6μ und der in pluri-lokulären entstandenen 8μ . Die Keimung der Zygoten verläuft ganz ähnlich wie die der Schwärmer. Aber in meinen Kulturen degenerierten die Keimlinge aus den Zygoten vor der Entwicklung der kriechenden Wurzeln. Die Textfig. 3 zeigt die verschiedenen Entwicklungsstadien der Keimung von Zygoten. Wie die Figuren zeigen verläuft die Entwicklung der Zygote zwischen *A* und *a* etwas schlechter als die der Zygote zwischen *B* und *b*.



Textfig. 2 *Heterochordaria abietina* Ungeschlechtliche Keimung der Schwärmer. *A-E* Keimlinge von Schwärmer aus *A*, *A'-E'* die von Schwärmer aus *a*, *A''-D''* die von Schwärmer aus *b* Schwärmer aus *b* nicht keimt Vergr. 660.

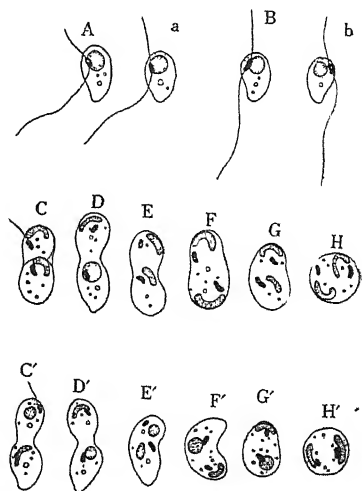


Textfig. 3. *Heterochordaria abietina* Keimlinge der Zygoten A-Q Keimlinge der Zygote zwischen A und a, A'-L' dieselbe zwischen B und b Vergr. 660

II SCYTOSIPHON LOMENTARIUS (LYNGB) AG

Bei dieser Alge ist nur das plurilokuläre Sporangium bekannt. Die Kopulation der in diesen Sporangien entstandenen Schwärmer wurde schon wiederholt von verschiedenen Autoren untersucht. BERTHOLD (1881) gibt an, dass er bei diözischen Pflanzen aus Neapel nur selten Kopulation fand. Nur einmal gelang es ihm, ein männliches Exemplar zu bekommen. KUCKUCK (1912) beobachtete bei Helgoland auch Kopulation der Schwärmer dieser Alge; er bekam aber höchstens eine Zygote auf 100 Schwärmer. Nach ihm sind die Pflanzen in dieser Gegend monözisch, und nur diejenigen Schwärmer, die früh morgens herauskamen, haben die Fähigkeit, zu kopulieren. An der marokkanischen Küste bei Tanger hat er auch Gelegenheit gehabt, diese Pflanzen zu sammeln. Aber die mit diesen Exemplaren angestellten Versuche waren ganz ergebnislos. Es gelang SAUVAGEAU (1928) nicht, an der französischen Westküste (Guéthary) Kopulation dieser Alge nachzuweisen. DAMMAN (1930) machte auch Versuche, in Helgoland Kopulation dieser Pflanze zu erzielen, aber hatte keinen Erfolg damit. Auch KYLIN (1933) hat bei dieser Pflanze in Kristneberge keine Kopulation beobachtet. Seiner Meinung nach ist die oben erwähnte Kopulation der Schwärmer bei dieser Alge keine echte sexuelle, sondern nur eine Scheinkopulation.

Um diese Erscheinung noch genauer zu erkennen, stellte ich einige Untersuchungen an. Unter den Exemplaren in Asamushi konnte ich gleich viererlei Individuen: *A*, *a*, *B* und *b* unterscheiden. *A* und *a* sind viel dicker als *B* und *b*. Aber *A* (bzw. *B*) sieht ziemlich stärker als *a* (bzw. *b*) aus (Taf. X, Fig. 2). Die Schwärmer aus diesen viererlei Individuen keimen bald ohne, bald nach Kopulation. Die Kopulation erfolgt nur zwischen *A* und *a*, und zwischen *B* und *b*. Die Schwärmer aus *A* sind etwas grösser als die aus *a*, ebenso die Schwärmer aus *B* grösser als die aus *b* (Textfigg. 4, *A*, *a*, *B*, *b*).

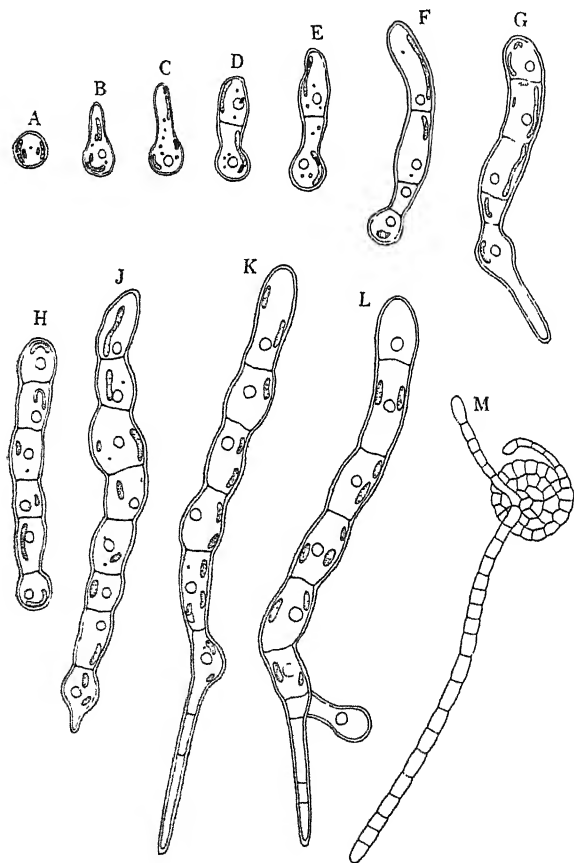


Textfig. 4. *Scytosiphon lomentarius*. Viererlei Schwärmer und ihre Kopulation. *A*, *a*, *B*, *b* Schwärmer aus Individuen *A*, *a*, *B* und *b* C-H aufeinanderfolgende Stadien der Kopulation zwischen *A* und *a*, C'-H' dieselbe zwischen *B* und *b*.

	Schwärmer aus <i>A</i>	aus <i>a</i>	aus <i>B</i>	aus <i>b</i>
lang :	7.1 μ	6.5 μ	6.6 μ	5.9 μ
breit :	3.8 μ	3.7 μ	3.6 μ	3.4 μ
abgerundet :	5.8 μ	5.3 μ	5.7 μ	4.8 μ

Die Kopulationsvorgänge verlaufen ganz ähnlich wie bei *Heterochordaria abietina* (Textfigg. 4, C-H, C'-H'). Die Zygote zwischen *A* und *a* hat einen Durchmesser von etwa 6.4 μ und die zwischen *B* und *b* einen von 6.1 μ .

Nach etwa 24 bis 30 Stunden beginnen die Schwärmer und die Zygoten zu keimen. Zuerst bildet sich ein kurzer Keimschlauch aus, und nach etwa zwei Tagen wird die erste Zellwand gebildet (Textfigg. 5, A-E). In den meisten Fällen entwickelt sich zuerst ein monosiphoner Zellfaden (Textfigg. 5, F-L). Mit fortschreitender Entwicklung bilden aber die Keimlinge eine Haftscheibe aus (Textfig. 5, M), an der sich endgültig aufrechte Pflänzchen entwickeln dürften.



Textfig. 5 *Scytosiphon lomentarius* Keimlinge der Zygote A-M Keimlinge der Zygoten zwischen *B* und *b*. Vergr. A-L 600, M 99.

Entwicklung der Zygote zwischen *A* und *a* etwas schlecht.

Wie bei *Heterochordaria* ist die Ent-

III. *SOROCARPUS UVAEFORMIS* (LYNGB.) PRINGSH

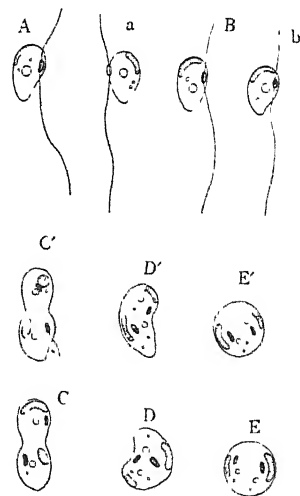
Sorocarpus uvaeformis ist eine Spezies der Ectocarpaceen. Erst ganz kürzlich wurde das Vorkommen dieser Alge an der nördlichen Küste Japans bemerkt. Wie bekannt, gibt es ziemlich viele Varietäten dieser Pflanze. Die für meine Untersuchung benutzten Pflanzen sehen etwas anders aus als die von OKAMURA (1929) seinerzeit in seiner Ikonographie abgebildeten.

Auch bei dieser Alge sind bis jetzt nur Individuen mit plurilokulären Sporangien bekannt. Aber ich konnte auch unter ihnen viererlei Individuen: *A*, *a*, *B* und *b* unterscheiden.

Die Messungen an den Schwärmern dieser Individuen ergaben folgende Werte.

	Schwärmer aus <i>A</i>	aus <i>a</i>	aus <i>B</i>	aus <i>b</i>
lang:	7.3 μ	6.1 μ	6.9 μ	6.4 μ
breit:	4.0 μ	3.9 μ	3.5 μ	3.8 μ
abgerundet:	6.5 μ	5.8 μ	6.5 μ	5.5 μ

Die Gestalt der Schwärmer ist der von *Heterochordaria* und *Scytosiphon* ähnlich (Textfigg. 6, *A*, *a*, *B*, *b*). Auch bei diesem Falle keimen die Schwärmer bald ohne, bald nach Kopulation. Nach etwa 24 Stunden beginnen die Schwärmer zu keimen und entwickeln dann einen Keimschlauch. Die erste Querwand ist schon nach zwei Tagen zu beobachten. In den meisten Fällen entwickelt sich zuerst ein monosiphoner Zellfaden. Mit fortschreitender Entwicklung fängt aber die Bildung der Seitenzweige an. Kopulation der Schwärmer erfolgt nur zwischen *A* und *a*, und zwischen *B* und *b* (Textfigg. 6, *C-E*, *C'-E'*). Die Zygote von *A* und *a* hat einen Durchmesser von etwa 8.0 μ und die von *B* und *b* einen von 5 μ . Die Keimung der Zygoten ist ebensowie die der Schwärmer.



Textfig. 6. *Sorocarpus uvaeformis*. Viererlei Schwärmer und ihre Kopulation. *A*, *a*, *B*, *b* Schwärmer von Individuen *A*, *a*, *B* und *b*. *C-E* aufeinanderfolgende Stadien der Kopulation zwischen *A* und *a*, *C'-E'* dieselbe zwischen *B* und *b*. Vergr. 1040.

ZUSAMMENFASSUNG

Bei *Heterochordaria abietina*, *Scytosiphon lomentarius* und *Sorocarpus wuaeformis* können wir viererlei Individuen (*A*, *a*, *B* und *b*) unterscheiden, die in ihrem Aussehen verschieden sind. Bei *Heterochordaria abietina* sind die Sporangien von *A* und *a* unilokulär und die von *B* und *b* plurilokulär, aber alle Sporangien bei *Scytosiphon lomentarius* und *Sorocarpus wuaeformis* sind plurilokulär. Die Schwärmer dieser Pflanzen fungieren fast alle sowohl ungeschlechtslich als auch geschlechtlich. Kopulation erfolgt nur zwischen *A* und *a*, und zwischen *B* und *b*. Bei diesen beiden Fällen ist der weibliche Gamet etwas grösser als der männliche. Kopulation unter den Schwärmern von gleichen Individuen kommt niemals vor. Also ist die Geschlechtstrennung bei diesen Algen stark fixiert. Die Individuen *A* und *a*, sehen immer kräftiger als *B* und *b* aus, meiner Meinung nach sind *A* und *a* diploid und *B* und *b* haploid. Die Chromosomenreduktion dürfte bei der ersten Kernteilung in den Sporangien von *A* und *a* erfolgen.

Sicheren Aufschluss über den Generationswechsel dieser Pflanzen werde ich bei nächster Gelegenheit durch eine zytologische Untersuchung zu erzielen suchen.

Herrn Prof. Dr. TAHARA, unter dessen Leitung diese Arbeit ausgeführt wurde, möchte ich hier meinen verbindlichsten Dank dafür aussprechen.

LITERATURVERZEICHNIS.

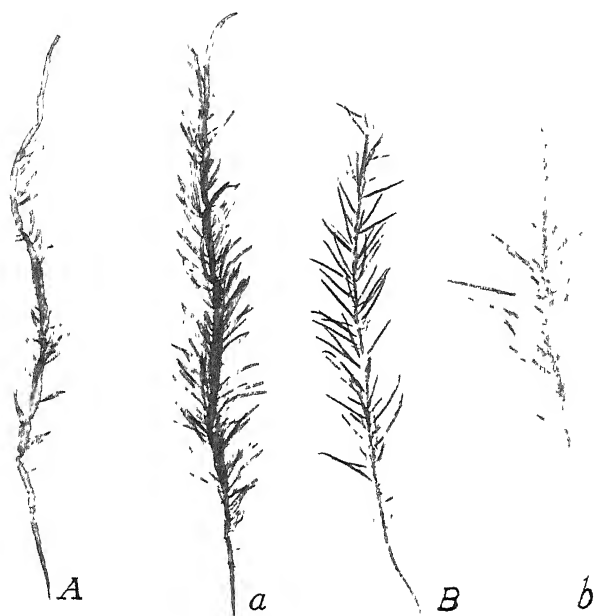
- 1) BERTHOLD, G. 1881. Die geschlechtliche Fortpflanzung der eigentlichen Phaeosporeen Mitt. aus der zool. Stat. zu Neapel, Bd. 2
- 2) CLINT, C. 1927. The life-history and cytology of *Sphacelaria bipinnata* SAUV. Univ. of Liverpool Publ. of the Hartley Bot. Laboratories, No. 3.
- 3) DAMMAN, H. 1930. Entwicklungsgeschichtliche und zytologische Untersuchungen an Helgolander Meeresalgen. Wiss. Meeresunter., N. F., Abt. Helgoland, Bd. 18.
- 4) IKARI, J. 1928. On the culture of Swarmspores of *Heterochordaria abietina* (RUPR.) S. et G. Bot. Mag., Tokyo. Vol. 42.
- 5) KNIGHT, M. 1923. Studies in the Ectocarpaceae I. The life-history and cytology of *Pylaiella littoralis* KJELLM. Trans. Roy. Soc. Edin., Vol. 53.
- 6) KNIGHT, M. 1929. Studies in the Ectocarpaceae II. The life-history and cytology of *Ectocarpus siliculosus* DILLW. Trans. Roy. Soc. Edin., Vol. 56.
- 7) KLUCKUCK, P. 1912. Die Fortpflanzung der Phaeosporeen. Wiss. Meeresunter., N. F., Abt. Helgoland, Bd. 5.
- 8) KYLIN, H. 1918. Studien über die Entwicklungsgeschichte der Phaeophyceen. Svensk bot. Tidskr., Bd. 12.

- 9) KYLIN, H 1933 Ueber die Entwicklungsgeschichte der Phaeophyceen Lunds Univ. Arsskr., N. F., Avd. 2, Bd 29, Nr. 7.
- 10) OKAMURA, K 1929. Icones of Japanese Algae Vol VI, No 1
- 11) OLTMANN, F. 1922 Morphologie und Biologie der Algen. 2. Aufl., Bd 2, Jena.
- 12) SAUVAGEAU, C. 1929 Sur le développement de quelques Phéosporées Bull. Stat. biol. d'Ancachon, T. 26, Bordeaux
- 13) SCHREIBER, E 1932. Ueber die Entwicklungsgeschichte und die systematische Stellung der Desmarestiaceen. Zeitschr. für Botanik, Bd 25

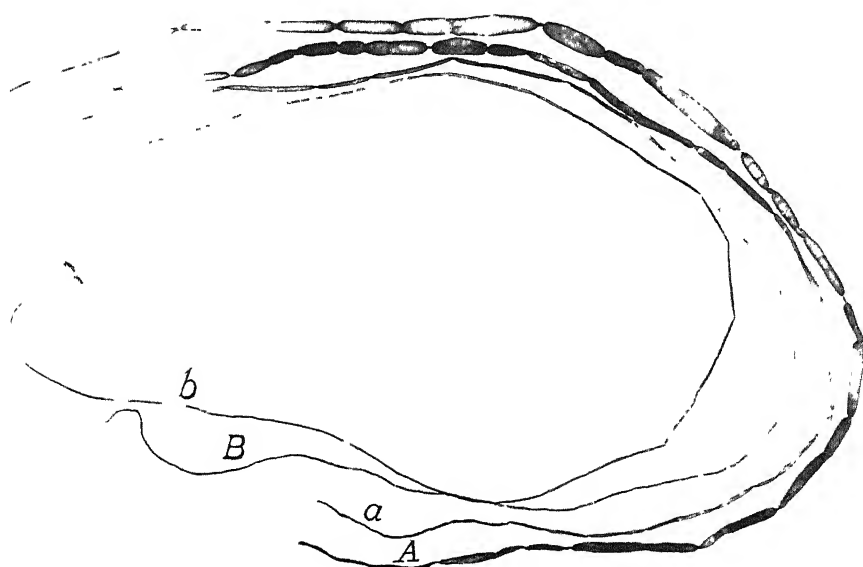
TAFELERKLÄRUNG

TAFEL X.

- Fig 1 *Heterochordaria abietina* Viererlei verschieden aussehende Individuen. A weibliches Individuum mit unilokularen Sporangien, a männliches mit denselben B weibliches Individuum mit plurilokularen Sporangien, b männliches mit denselben Verkl. 4/5
- Fig 2 *Scytosiphon lomentarius*. Viererlei verschieden aussehende Individuen. A, B weiblich, a, b männlich. Verkl. 2/5.



1



2

ON THE GROWTH OF THE SHELL OF *MERETRIX MERETRIX*,
ESPECIALLY WITH REGARD TO PERIODICITY OF
GROWTH RELATIVELY TO THE SEASONAL
VARIATION IN THE ENVIRONMENT

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(With five figures in text)

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In the formula of relative growth, $y=ax^b$, the signification of the constant a has been examined by NOMURA (1926, '28), and its modification a_m has been given by HAMAI (1934), as the local constant. The present writer understands the constant b as being the ratio between the two constants, which are to be explained later.

The constant b signifies naturally a condition of relative growth between the two dimensions, x and y , and varies with locality and season (time phase). In my previous paper, however, the relation between these constants and the environmental conditions has not been discussed in detail. The purpose of the present paper is to state and explain these details and to elucidate the features of the natural growth in the shell of *Meretrix meretrix* with regard to the various values of the constant b .

This investigation has been carried under the cordial guidance of Prof. E. NOMURA, to whom the present writer wishes to express his sincere thanks.

MATERIAL AND METHOD

The collection of specimens of *Meretrix meretrix* was carried out in the sea inside the breakwater in Kusatu, Hiroshima, where native fishermen usually raise this clam for commercial purposes. All the specimens were obtained only from a definite area, of about 100 square metres, in order to limit the change in the environment to definite conditions, and, in order to make the monthly interval between the consecutive collections equal as much as possible, they were made on the 14th of every month from October, 1932, to September, 1933. They have been preserved in a 3 per cent. formalin solution of sea water.

The specimens thus collected included naturally animals of various ages. Their linear dimensions, viz. length, depth and height, and their shell weight including ligament only, were measured. Both the methods of measurement and of calculation were the same as those stated in my previous paper (1934).

RELATION BETWEEN THE TIME AND THE GROWTH OF MERETRIX MERETRIX

ROBERTSON (1908, '23), BRODY (1921) and others applied to the growth of the organisms the equation of an autocatalytic mono-molecular reaction, viz.

$$\log \frac{x}{A-x} = K(t-t_1) \dots \dots \dots (1)$$

where x is the weight of organism at time t , A the final weight attainable, t_1 the time when $x=A/2$, and K the growth constant. The equation represents a sigmoid curve of growth, and, when this curve is to be repeated twice or more, the following formula is adopted:

$$\log \frac{x-w}{A-(x-w)} = K(t-t_1) \dots \dots \dots (2)$$

where w is the weight gained until the beginning of the growth cycle under consideration, and A is the total weight gained during the cycle, the other notations remaining the same as in (1).

Formulae, (1) and (2), have an inflection point at $A/2$ or t_1 . To the animals, in which the growth curve has the inflection point below $A/2$, ROBERTSON (1926) applied the modified formula:—

$$\log \frac{x+B}{A-x} = K(t-t_1) \dots \dots \dots (3)$$

and indicated that the degree of asymmetry can be calculated by the constant B . BRODY (1926) also represented the growth, after the inflection point, by the equation

$$W = A - Be^{-kt} \dots \dots \dots (4)$$

where W denotes the body weight at age t , e the base of natural logarithms, and k the fractional decline of growth, which is termed the specific velocity constant in the publications and reports on chemical kinetics. B is a parameter, the value of which increases with the gain in length of the process, which precedes the point of inflection in the phase under consideration. Moreover, BRODY in relation to the formula (4) stated

that the limiting value A may, for practical purposes, be considered as denoting the mature weight of the animal, which is its genetic growth constant. When the animal grows normally under given favourable conditions, this mature weight ought to be a characteristic of its own species.

According to BRODY, in the process of growth there are two independent phases, the self-accelerating and the self-inhibiting. Equation (4) was adopted for the latter phase, after the point of inflection on the growth curve, and its velocity of growth declines in a geometrical progression with age. In the case of the self-accelerating phase BRODY used

$$W = Ae^{kt} \dots \dots \dots (5)$$

where A is the weight at the beginning of the entire growth period. If this is transformed into logarithms, then

$$\log W = \log A + kt$$

On differentiating this

$$\frac{d \log W}{dt} = k$$

The relative growth rate is therefore constant.

These equations of BRODY's, however, are not sufficiently based upon the biological conception, as has also been pointed out by KAUFMAN (1930).

During the complete life cycle of the organisms, the activity of both acceleration and inhibition may function in the growth of the body as internal factors in its continuous discharging, and may change its features together with its regulation, which depends upon the environmental conditions when considered as a function of the external limiting factors. These natural processes may sustain the continuity of growth in so far as no sudden change in these internal and external factors occurs. With this consideration, how the external limiting factors determine the growth process as environmental conditions, and what features are given to the growth may form part of subject of investigation.

In the shell growth of *Meretrix meretrix*, it has first to be taken into account, as an internal physiological process, that the increase of the shell material depends upon the secretion of organic and inorganic substance, for instance, chitin and calcium carbonate, from the epithelium of the mantle. The explanation of the mechanism of this secretion must be a fundamental solution in connexion with the development of the shell, but an analysis of natural growth with special reference to the environmental conditions may be also contributive to the study of internal factors.

The applicability of (1) and (2) with regard to the growth of *Meretrix*

meretrix may possibly be confirmed by calculations, based on the data given by NAITÔ (1930) The results of calculations are shown in Table 1

TABLE 1.

Growth cycle	Date of observation	No. of specimens observed	t in day	Length in cm			Height in cm			Weight in gm		
				Average observed value	Calculated value	Difference	Average observed value	Calculated value	Difference	Average observed value	Calculated value	Difference
2nd cycle	Apr 27, 1925	45	0	2.00	2.01	0.01	1.75	1.75	0.00	2.20	2.18	-0.02
	May 27	45	30	2.10	2.09	-0.01	1.80	1.81	0.01	2.60	2.45	-0.15
	Jun. 28	44	62	2.27	2.27	0.00	1.94	1.95	0.01	3.31	3.13	-0.18
	Jul 21	44	85	2.47	2.48	0.01	2.12	2.10	-0.02	4.18	3.99	-0.19
	Sept 9	44	135	2.88	2.93	0.05	2.42	2.46	0.04	6.36	6.52	0.16
	Oct 8	40	164	3.10	3.08	-0.02	2.59	2.59	0.00	7.82	7.59	-0.23
	Nov. 8	39	195	3.17	3.15	-0.02	2.68	2.67	0.01	8.40	8.19	-0.21
	Dec 9	37	226	3.18	3.18	0.00	2.71	2.70	-0.01	8.51	8.45	-0.06
	Jan. 7, 1926	41	255	3.19	3.19	0.00	2.71	2.71	0.00	8.52	8.53	0.01
3rd cycle	Feb 5	41	284	3.19	3.19	0.00	2.71	2.71	0.00	8.56	8.59	0.03
	Mar. 8	33	315	3.19	3.21	0.02	2.71	2.72	0.01	8.39	8.70	0.31
	Apr. 18	35	356	3.20	3.27	0.07	2.71	2.76	0.05	8.77	9.13	0.36
	May 30	33	398	3.45	3.42	-0.03	2.90	2.87	-0.03	10.70	10.33	-0.37
	Jun. 28	33	427	3.62	3.61	-0.01	3.03	3.00	-0.03	12.21	11.92	-0.29
	Jul 29	33	458	3.81	3.86	0.05	3.14	3.17	0.03	13.45	14.23	0.78
	Aug. 29	33	489	4.06	4.10	0.04	3.32	3.33	0.01	16.06	16.46	0.40
	Oct. 10	34	531	4.30	4.29	-0.01	3.48	3.47	-0.01	18.64	18.36	-0.28
	Nov. 9	34	561	4.38	4.36	-0.02	3.53	3.51	-0.02	19.06	18.98	-0.08
	Dec. 11	34	593	4.39	4.39	0.00	3.53	3.53	0.00	19.22	19.28	0.04
	Jan. 28, 1927	34	641	4.40	4.40	0.00	3.54	3.55	0.01	19.38	19.44	0.06
4th cycle	Feb. 24	33	668	4.41			3.55			19.69		
5th cycle	May 7, 1928	8	1106	5.56			4.33			35.00		

and are plotted in Fig. 1. This growth cycle is repeated with a period of one year, and the velocity of growth in weight increases, and that in length and height decreases, with age at the same time phase in each annual cycle.

According to NAITÔ, the data shown in Table 1 were originally obtained from monthly measurements of the specimens reared in Kankawa, Tiba, in a wire-gauge cage embedded in the sand. This cage was about 30 cm. in length and breadth and about 36 cm. in height, having meshes of about 9 mm. in the floor and on the sides and of about 12 mm. in the roof. At first, on April 27, 1925, 45 young clams, which had been developed in 1924, were placed in this cage. The measuring of these clams was therefore begun in the second cycle of growth of this bivalve.

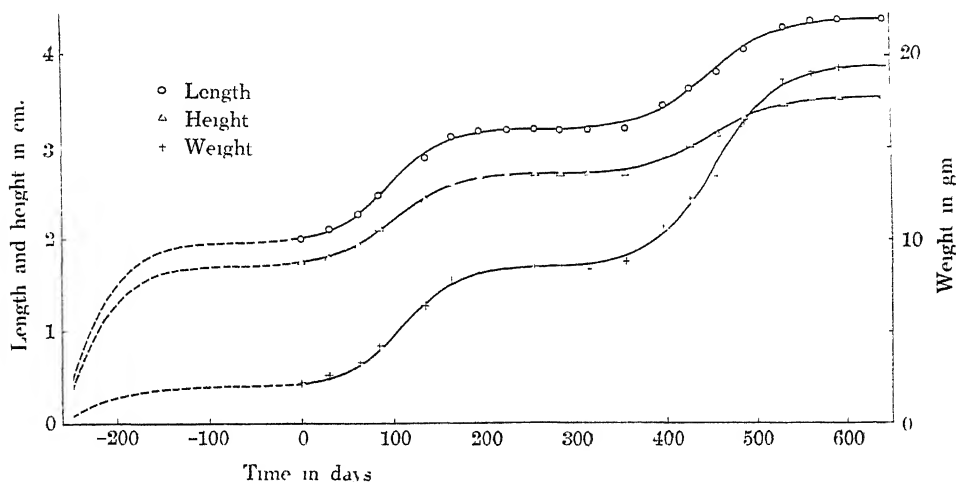


Fig. 1. Curves expressing growth of *Meretrix meretrix*. Points represent actual measurements, and thick lines loci of calculated values.

Small clams of less than 5 mm. in length are found in abundance every year on sandy sea shores from the end of August to the beginning of September. From this fact in connexion with the spawning period, which will be described later, the part of the broken lines in Fig. 1 may possibly be deduced assuming a similarity of this growth curve to those of the succeeding growth cycle, that is to say, if the formation of the shell commences in August, in which the veliger larvae attain the shape of the adult.

In calculating NAITÔ's data, the following formulae were obtained :

$$\left. \begin{array}{l} \text{Length, 2nd cycle : } \log \frac{x-1.95}{1.25-x} = 0.0140 (t-95) \\ \text{3rd cycle : } \log \frac{x-3.18}{1.23-x} = 0.0120 (t-450) \end{array} \right\}$$

$$\left. \begin{array}{ll} \text{Height, 2nd cycle:} & \log \frac{x-1.70}{1.02-x} = 0.0131 (t-100) \\ & \text{3rd cycle:} \quad \log \frac{x-2.70}{0.85-x} = 0.0118 (t-150) \\ \text{Weight, 2nd cycle:} & \log \frac{x-2.00}{6.60-x} = 0.0110 (t-111) \\ & \text{3rd cycle:} \quad \log \frac{x-8.50}{11.00-x} = 0.0123 (t-155) \end{array} \right\} \dots (6)$$

RELATIVE GROWTH-RATE AND ITS RATIO

The decline in the rate of growth may be observed in the relative rate of growth. The absolute velocity of growth (v) is obtained in differentiating formula (1), viz.

$$v = \frac{dx}{dt} = kx(A-x) \quad \dots \dots \dots (7)$$

where $k=K/A$. By determining dx/x in percentage of x , and when this percentage is rearranged, the following formula is obtainable

$$\frac{dx}{x \cdot dt} = \frac{d \log x}{dt} \quad \dots \dots \dots (8)$$

as the relative rate of growth.

When the relative rates of growth in (6) are plotted against t (time or age), the curves in Fig. 2 are obtained, and from these, in each dimension and in the weight, it is clearly recognizable that the relative growth rate declines with the increase in the number of years.

MINOT (*vide* THOMPSON 1917 and NEEDHAM 1931) concluded that the rate of senescence is greater in the earlier stage, from the fact of the decline in relative growth rate taken as the increment in percentage of the weight of the animal at the beginning of the period in question. THOMPSON (1917) criticised this conclusion and stated that the application of $\frac{dx/x}{dt}$ for animal growth could only be justified, if it expresses a straight line or some other curves simpler than the usual growth curves. The decline in the relative growth rate with age is, however, a biological fact, and its comparison among organs or animals must be useful.

When the points at the same time phase in each cycle, viz. A-A or B-B, on each curve of relative growth rate in Fig. 2 are connected, they

make a hyperbolic curve, which may be expressed approximately by the formula

$$\frac{dx/x}{dt} = \frac{\alpha}{t} \quad \dots \dots \dots (9)$$

where α is a constant.

MURRAY (1926) has applied the formula (9) to the growth of the chick embryo and found it to fit the case. SCHMALHAUSEN (1927, '29, '31) derived the formula (9) independently from his own study of the growth in the volume of the chick embryo, and, from the fact that the relative growth rate is inversely proportional to time, he established the law of growth, in which he urges that "Das Produkt aus Wachstumsgeschwindigkeit (relative growth rate) und Zeit hat bei konstanten Bedingungen des Wachstums einen konstanten Wert."

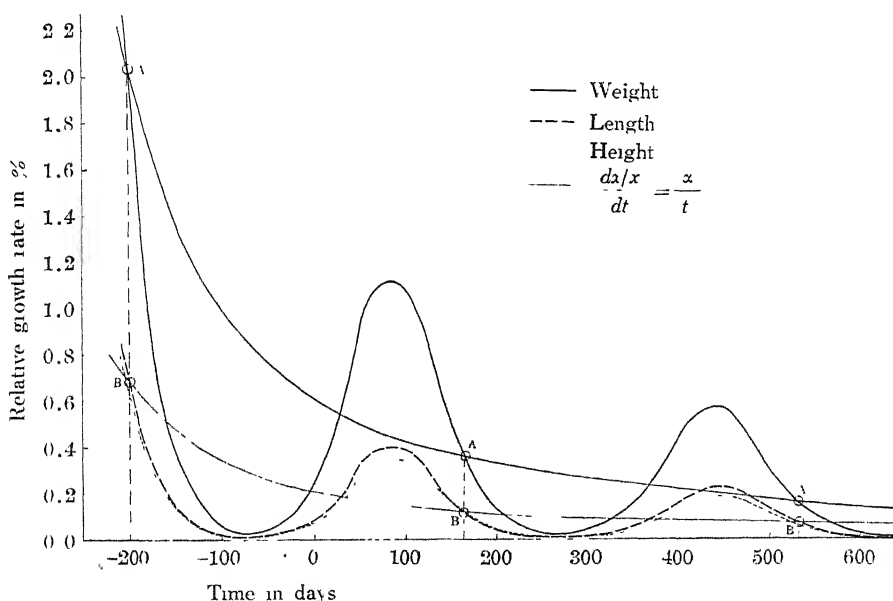


Fig. 2. Heavy line and broken lines represent daily change of relative rate of growth, and thin lines annual change expressed by (9)

Also in the present investigation, the decline of relative growth rate with the prolongation of age in a certain time phase or in a certain environmental condition, which is repeated every year in the same manner, conforms to the law of growth of SCHMALHAUSEN. α in the formula (9) is, then, an index, which shows the decline in the constant environmental

conditions, and it may be called the *conditional growth constant*.

By integrating (9), we obtain

$$x = C_1 t^{\alpha} \quad (10)$$

In the case of the other dimension y , similarly

$$y = C_2 t^{\beta} \quad (11)$$

From (10) and (11)

$$y = ax^b \quad (12)$$

is obtained, where $b = \beta/\alpha$, and $a = C_2/C_1^b$. Therefore, b is the ratio between the two conditional growth constants, β and α , or the ratio between the relative growth rates of the two dimensions, y and x , as stated at the beginning of this paper.

HUXLEY (1931) derived the same equation as (12) from the equations,

$$\frac{dy}{dt} = \beta y G \quad \text{and} \quad \frac{dx}{dt} = \alpha x G$$

in which β and α are the specific constants, which are defined respectively for a certain organ and for the rest of the body; and G measures the general condition of growth, as affected by age and environment. Since however G has a broad meaning, the formula (12) can be derived from simple exponential formulae as well as from the parabolic formulae, viz. (10) and (11), as already pointed out by SCHMALHAUSEN (1931).

PERIODICITY OF THE SHELL GROWTH

The monthly variations in the value of b in connexion with my monthly collections of *Meretrix meretrix*, i.e. the changes of b at different time phases, indicating the periodical change which makes an annual circulation, are shown in Table 2, and these records are plotted in Fig. 3.

Weight-length relation. The real value of b gradually showed an increasing tendency from February to July. After reaching its maximum in July, it decreased considerably in August, and again increased in September, and then gradually showed a decreasing tendency until February.

Height-length relation. b in this relation remained generally constant throughout the year, although it might be shown that its value was higher in summer than in winter.

Depth-length relation. In this relation, the lowest value was found in June, and the highest in September; and the rapid change appeared from July to September. In other words, the relative growth of depth was

TABLE 2.

Month	No of specimens	Depth-length Relation		Height-length Relation		Depth-height Relation		Weight-length Relation	
		b	a	b	a	b	a	b	a
Oct., 1932	147	1.05	0.471	0.90	0.943	1.17		2.58	0.212
Nov.	142	1.07	0.463	0.92	0.912	1.16		2.55	0.213
Dec.	159	1.03	0.484	0.87	0.972	1.18		2.54	0.227
Jan., 1933	167	1.04	0.478	0.89	0.947	1.17		2.50	0.234
Feb.	159	1.02	0.490	0.89	0.949	1.15		2.51	0.236
Mar.	147	0.99	0.509	0.89	0.944	1.11		2.51	0.236
Apr.	145	1.01	0.500	0.91	0.923	1.11		2.50	0.237
May	132	0.98	0.514	0.89	0.945	1.10		2.56	0.214
Jun.	90	0.96	0.528	0.94	0.898	1.02		2.63	0.199
Jul.	116	0.97	0.518	0.90	0.954	1.08		2.77	0.182
Aug.	138	1.03	0.486	0.92	0.913	1.12		2.63	0.194
Sept.	148	1.08	0.454	0.92	0.913	1.17		2.74	0.167

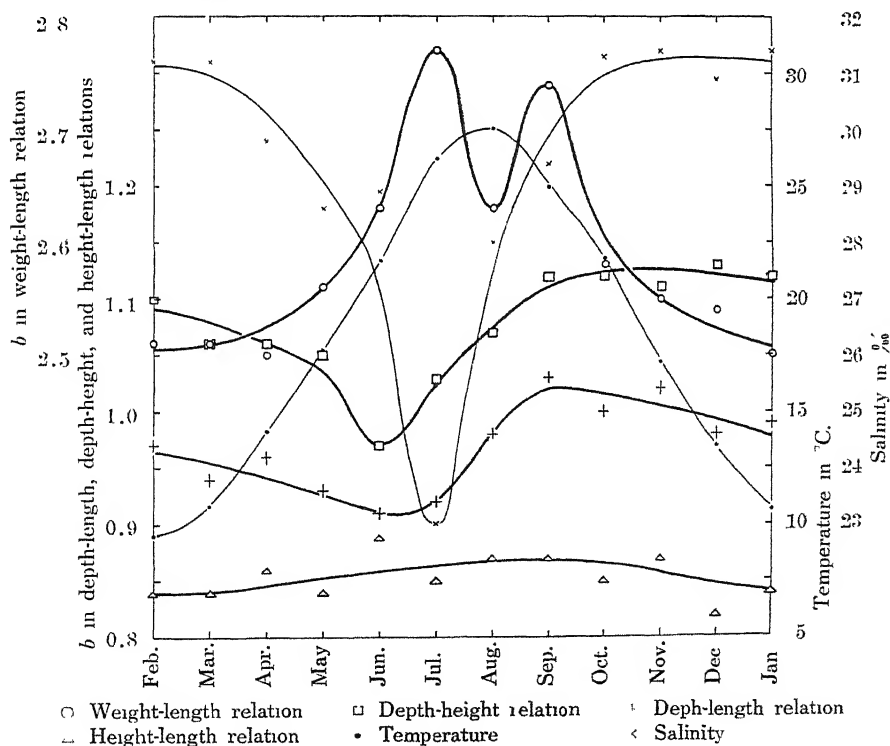


Fig. 3. Curves to show seasonal variation of constant b (Table 2), and of temperature and salinity (Table 3).

smaller than that of length from March to July, and during this period, the ratio between their rates descended gradually and began to ascend rapidly from July to September. In the period from August to February, the rate of depth was larger than that of length, and, within this period, the ratio lowered gradually from September to February.

From the point of view that the increase of depth depends originally upon the concentric increment along the shell edge, which is to grow usually in the direction of length and of height, the low relative growth rate of depth to length in July and the sudden rise until September may perhaps suggest an alteration in the angle formed by the shell edge and the plane of the meeting of the edges.

THIEL (1926) observed such an alteration of the angle in *Sphaerium corneum* according to the difference in the degree of increment in the height or in the depth of the shells from different localities having different environmental conditions as, for instance, the difference in the oxygen content in the medium.

The height and length of the bivalves show generally a similar relation to the depth. In order to simplify the matter and to compare with THIEL's observation, the curve of monthly change, which expresses the depth-height relation, is also shown in Fig. 3 together with other curves, the depth-height relation being derived from the height-length and the depth-length relations. Thus the depth-height relation reveals a tendency to change similar to that of the depth-length relation.

In both the depth-length and the depth-height relations the changes in the value of b may be divided into two parts, viz one, the period from February to August, during which the value is relatively lower, and the other, the period from August to February, during which the value is relatively higher. For convenience, let us call the former the *spring-summer period*, and the latter the *autumn-winter period*. In the spring-summer period, the increment along the shell edge is elongated comparatively towards the outward direction, and in the autumn-winter period comparatively towards the inward direction, relatively to the plane where both valves meet each other. In other words, in the spring-summer period the shell grows larger antero-posteriorly and dorso-ventrally, this resulting in the comparatively lower value of b , and in the autumn-winter period the shell becomes larger dextro-sinistrally, this resulting in the comparatively higher value of b . This dextro-sinistral thickening may probably be taken as the development in bulk or the *fattening* of the shell.

When the asymmetrical angular change, which is caused by an asym-

metrical addition of shell material, occurs on the valves of the shell, it may give rise to a sort of asymmetrical growth as in the case of *Sanguinolaria olivacea* JAY. NOMURA (1933) has shown that this asymmetry is revealed by the difference in *b*. That is to say that in those bivalves larger than 63 mm. in length, the left valve tends to have larger growth rate than the right valve, and hence the depth of the left valve becomes deeper than that of the right in order to make a close contact of the edges of both valves with each other. This kind of asymmetrical growth may be caused by the change of internal factors rather than that of environmental conditions.

The breeding season of *Meretrix meretrix* lasts from May to September in Kankawa. According to NAITÔ, mature eggs and sperm are found in May. The spawning begins at the end of June, and is most vigorous during July, and then becomes less vigorous at the beginning of August. After this, even, specimens which contain mature germ cells may be found, but the spawning becomes very scant, and in the middle of August it is over. In the beginning of September, specimens which contain germ cells are again found, but these germ cells do not mature. The temperature of sea water ranges roughly from 23°C. to 27°C. in the most vigorous period of spawning. The spawning period of the present species may thus be included in the latest part of the spring-summer period, and it may be supposed that a certain relation should exist between the spawning period and the rhythmic growth of the shell, but this relation can hardly be definitely stated at present because of the scanty data.

PROBABLE RELATION BETWEEN TEMPERATURE AND THE RATIO OF RELATIVE GROWTH RATE

CROZIER (1926) has shown that the velocity of growth, taken as the reciprocal of time required to attain a given size or stage of development, is in accordance with ARRHENIUS' equation in connexion with temperature.

It is evident that the relative growth rate of *Meretrix meretrix* is greater in the higher temperature of summer (Table 3), as shown in Fig. 4. In general, the relative growth rate of the second growth cycle is higher in each dimension than that of the third cycle, even in the same temperature. This fact may be considered to be the effect of age. Furthermore, the relative growth rate in the ascending temperature is higher than that in the descending temperature. Thus, we may consider it as due not only to the effect of the difference in the time of the same

TABLE 3.

Month		Kankawa, Tiba		Kusatu, Hiroshima	
		Average Temperature	Average Salinity	Average Temperature	Average Salinity
Jan	Upper part	6.90	29.13	10.63	31.36
	Middle part	5.95	30.35		
	Latter part	5.00	32.63		
Feb.	Upper part	4.70	31.91	9.50	31.24
	Middle part	5.90	32.30		
	Latter part	5.15	32.38		
Mar	Upper part	6.45	30.79	10.77	31.23
	Middle part	8.50	30.01		
	Latter part	10.10	28.06		
Apr.	Upper part	12.23	31.83	11.05	29.78
	Middle part	13.30	31.33		
	Latter part	15.57	28.87		
May	Upper part	16.93	28.53	17.67	28.62
	Middle part	17.43	28.99		
	Latter part	20.07	27.11		
Jun.	Upper part	21.10	26.66	21.63	28.86
	Middle part	22.70	28.00		
	Latter part	23.50	28.95		
Jul	Upper part	25.77	26.87	26.22	23.01
	Middle part	26.93	25.50		
	Latter part	27.30	26.27		
Aug	Upper part	29.13	21.51	27.51	27.97
	Middle part	28.50	25.18		
	Latter part	28.33	23.35		
Sept	Upper part	26.07	22.58	25.16	29.10
	Middle part	21.60	23.37		
	Latter part	22.10	26.65		
Oct	Upper part	21.00	27.07	21.92	31.33
	Middle part	19.07	28.87		
	Latter part	17.03	30.10		
Nov.	Upper part	16.67	28.73	17.21	31.36
	Middle part	14.70	30.25		
	Latter part	13.10	30.81		
Dec	Upper part	11.73	31.07	13.54	30.91
	Middle part	10.40	30.84		
	Latter part	7.40	30.86		

growth cycle, but also to other seasonal variation of limiting factors of growth in the environment with the exception of the temperature.

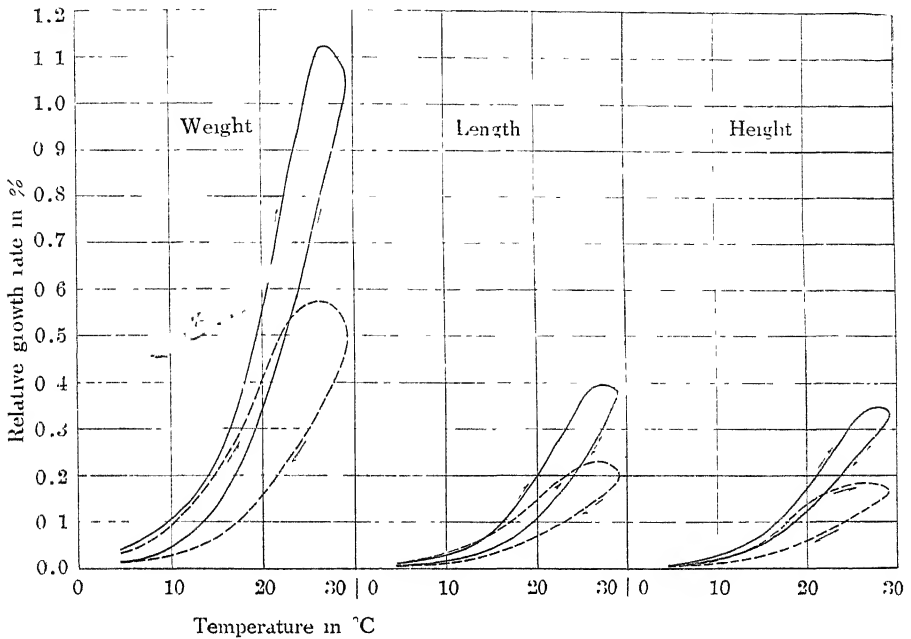


Fig. 4 Curves showing relative rates of growth. Relative rates calculated from formulae of (6) on ordinate, and temperature from Table 3 on abscissa. Second growth cycle in full lines, and third cycle in broken lines

The curves, which express the probable relations between temperature and the ratio of relative growth rate, are shown in Fig. 5.

Weight-length relation. The maximum value of the ratio b was found in a temperature of about 26.2°C ., and the ratio tended to decrease gradually with the lowering temperature below, but it tended to decrease rapidly with the raising of the temperature above, 26.2°C .. When the increase of the ratio is inhibited at a high temperature with a slight retardation of the relative growth rate (Fig. 4), it is natural to consider that the relative rate of increase in the weight of the shell may reach the maximum value at about 26.2°C ., as the average temperature and tends to decrease at a higher temperature than this, i.e. the growth in weight may be most active at about 26.2°C ., and may be retarded at a temperature higher than this. Consequently, the shell may become thicker at a higher than at a lower temperature.

Height-length relation. b in this relation, roughly speaking, kept nearly a constant value during the year, even though a tendency to increase with a rise of temperature was also recognizable.

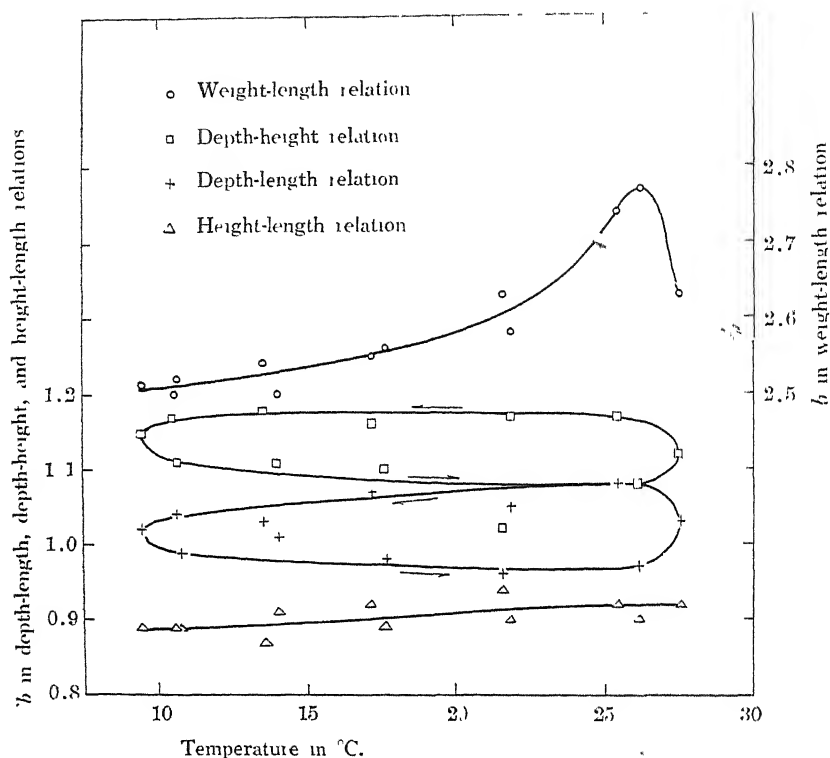


Fig. 5 Curves expressing the relations between temperature and the ratio of the relative growth rate.

Depth-length relation. In the rising temperature from February to August the relative growth rate of depth was comparatively lower than that of length, and in the falling temperature from August to February it was comparatively higher than that of length.

If the points are connected in the order of the time phase, a circulatory path may be drawn (Fig. 5). This circulatory path shows that the ratio of the relative growth rate of depth to that of length circulates with the monthly alteration of temperature, having the inflection point at the lowest temperature of February and at the highest temperature of August, and reveals evidently that there are spring-summer and autumn-winter periods.

Depth-height relation. This relation was almost similar to the depth-length relation.

The above conclusions may be summarized up as follows:

Each measurement of the shell of *Meretrix meretrix* shows, respectively, a different feature of growth, even it is slight. Consequently, the ratio b varies with the change of season or the time phase, and it may be supposed that the relative growth rate of each measurement is different, even in the same temperature of the two periods of growth.

WEYMOUTH, McMILLIN and RICH (1931) have observed that the initial rate of relative growth per year in the razor clam, *Siliqua patula*, is greater in a lower latitude than in a higher along the Pacific coast of North America, from Chiguik Bay, Alaska, to Pismo, California, and that the rate in the second year is greater in the north. In their research, the growth curve

$$L = Be^{-e^{-kt}}$$

has been used, in which L is the length at the time t , e the base of natural logarithms, and B , c and k the constants. This also suggests that the relative growth rate is related to temperature in some way

The relation between temperature and the local variation in *Meretrix meretrix* has been investigated by HAMAI (1934), and it is reported that the species was well grown especially at about 17.6°C. in annual average of temperature.

ORTON (1928) has studied the periodicity of growth. According to him, the growth period of *Ostrea edulis* occurs twice a year. One period begins in spring from April to May, when the temperature of the sea water is 50°-52°F., but the growth ceases before the breeding season, which begins when the temperature rises above 60°F in summer. The other period of growth begins when temperature falls below 60°-57°F. Furthermore, the growth of the gonad occurs in the spring growing period, and the fattening, i. e. the accumulation of reserve food products, mainly in the form of glycogen, occurs in the autumn growing period. In the present case of *Meretrix meretrix*, the gonad growing and spawning also appear in the spring-summer period, and the fattening in the autumn-winter period.

PROBABLE RELATION WITH SALINITY

In Kankawa, salinity is lower in July, August and September, and in

these months the relative growth rate is higher. In Kusatu, salinity is the lowest in July, and in this month, the ratio of the relative growth rate of weight to length is the highest, and that of depth to length is low. It may therefore be supposed that salinity affects growth in some way. ORTON (1925, '28) is of opinion that the shell growth of *Ostrea edulis* is due to, or favoured by, lower salinity

SUMMARY

1) A study has been carried out of the monthly collections of *Meretrix meretrix* in order to determine the true nature of its growth.

2) In the formula, $y = ax^b$, the constant b is the ratio between two conditional growth constants or between two relative growth rates, at the same time phase on each cycle of growth.

3) The natural growth of *Meretrix meretrix* can be expressed by the so-called logistic curve of ROBERTSON

4) The constant b shows also seasonal variations, which can be divided into two parts, viz. in *Meretrix meretrix*, the spring-summer period from February to August and the autumn-winter period from August to February.

5) The relative growth rates and their ratios in depth, height, length and weight are different even in the same temperature of the two periods.

6) The ratio of the relative rates of growth between weight and length showed the maximum value to exist at a temperature of about 26.2°C.

7) b in the depth-length and the depth-height relations makes a circulatory path with the monthly variations of temperature, having two inflection points, one in the lowest temperature of February and the other in the highest temperature of August.

8) The growth angle at the shell edge alters so as to be narrower from February to August and wider from August to February.

WORKS AND PUBLICATIONS REFERRED TO

- BRODY, S. 1921. The Rate of Growth of the Domestic Fowl. J. Gen. Physiol., 3.
 BRODY, S. and RAGSDALE, A. C. 1921. The Rate of Growth of the Dairy Cow. Extra-uterine Growth in Weight. J. Gen. Physiol., 3.
 BRODY, S. 1926. Time Relations of Growth. I. Genetic Growth Constants of Animals. J. Gen. Physiol., 8.
 CROZIER, W. J. 1926. On Curves of Growth, Especially in Relation to Temperature. J. Gen. Physiol., 10.
 HAMAI, I. 1934. On the Local Variation in the Shell of *Meretrix meretrix* (L.) with Special

Reference to Growth of Organism Sci Repts Tôhoku Imp Univ, Biol, 9 No. 2-3

- HUXLEY, S 1931 Problems of Relative Growth London
- KAUFMAN, L. 1930. Innere und ausserer Wachstumsfaktoren Untersuchungen an Hühnern und Tauben Arch f Entwicklungsmechn, 122
- MURRAY, H A 1926. Physiological Ontogeny J Gen Physiol, 9
- NAITÔ, S 1930 The Growth-rate of *Tapes philippinarum* and *Meretrix meretrix* Reports of the Naiwan Branch Station of the Tiba Fishery Institute 1930 (in Japanese).
- NAITÔ, S 1930 Inquiry on the Breeding Season in Mussels Reports of the Naiwan Branch Station of the Tiba Fishery Institute 1930 (in Japanese)
- NEEDHAM, J. 1931. Chemical Embryology Cambridge Vol I. Section 2 On Increase in Size and Weight
- NOMURA, E. 1926 Further Studies on the Applicability of $a-kb^k$ in Expressing the Growth Relations in Molluscan Shells Sci Repts Tôhoku Imp Univ, Biol, 2
- NOMURA, E 1928 On the Local Variation in Some Littoral Gastropods Sci Repts Tôhoku Imp Univ, Biol, 3
- NOMURA, E 1933. On the Asymmetrical Growth in the Shell of *Sanguinolaria olivacea* JAY Sci Repts Tôhoku Imp. Univ, Biol, 8
- ORTON, J H 1925 The Conditions for Calcareous Metabolism in Oysters and Marine Animals Nature, 116.
- ORTON, J H. 1928 On Rhythmic Periods in Shell-growth in *O edulis* with a Note on Fattening. J Mar Biol Assoc U K, 15
- ROBERTSON, T B 1908 On the Normal Rate of Growth of an Individual, and its Biochemical Significance Arch f Entwicklungsmechn, 25
- ROBERTSON, T B. 1923. Chemical Basis of Growth and Senescence Philadelphia and London
- ROBERTSON, T. B 1926. The Analysis of the Growth of the Normal White Mouse into its Constituent Processes. J Gen Physiol, 8.
- SCHMALHAUSEN, I 1927 Beitrage zur quantitativen Analyse der Formbildung I II. Arch. f. Entwicklungsmechn, 109, 110
- SCHMALHAUSEN, I 1929 Zur Wachstumstheorie Arch. f. Entwicklungsmechn., 116.
- SCHMALHAUSEN, I. 1931 Das Wachstumsgesetz als Gesetz der progressiven Differenzierung. Arch f Entwicklungsmechn, 123.
- THIEL M E. 1926 Formwachstumsversuche an *Sphaerium corneum*. Arch. f. Entwicklungsmechn, 108
- THOMPSON, D'ARCY, W 1917 Growth and Form Cambridge
- WEYMOUTH, F W, McMILLIN, H. C and RICH, W H. 1931 Latitude and Relative Growth in the Razor Clam, *Siliqua patula* J Exper. Biol, 8.

TABLES AS THE BASES OF CALCULATION

TABLE 4. October 11, 1932

Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Length in cm.	Depth in cm.	Height in cm.	Weight in gm.
2.27	1.10	1.96	1.5	3.36	1.78	2.87	5.0
2.29	1.10	2.00	1.9	3.38	1.71	2.79	4.5
2.40	1.19	2.07	2.0	3.40	1.80	2.87	5.3
2.41	1.13	1.98	1.6	3.43	1.74	2.76	5.3
2.48	1.23	2.17	2.1	3.43	1.70	2.87	5.5
2.62	1.26	2.20	2.4	3.43	1.69	2.82	5.1
2.67	1.39	2.30	2.6	3.43	1.76	2.95	5.6
2.74	1.31	2.30	2.7	3.45	1.74	2.80	5.4
2.74	1.38	2.29	2.8	3.45	1.78	2.91	5.8
2.75	1.35	2.23	2.5	3.47	1.81	2.92	4.4
2.80	1.45	2.45	3.3	3.48	1.69	2.83	5.1
2.84	1.47	2.50	4.1	3.48	1.62	2.81	5.0
2.86	1.41	2.11	2.7	3.49	1.75	2.78	4.6
2.90	1.41	2.54	3.6	3.49	1.75	2.93	5.3
2.90	1.46	2.46	3.4	3.50	1.70	2.84	4.7
2.91	1.43	2.45	3.1	3.51	1.81	2.94	4.6
2.92	1.40	2.59	3.2	3.51	1.85	2.88	6.5
2.95	1.48	2.48	3.6	3.52	1.73	2.90	5.3
3.00	1.46	2.50	3.7	3.53	1.74	2.89	4.9
3.00	1.50	2.51	3.0	3.53	1.71	2.99	5.8
3.00	1.41	2.53	3.3	3.53	1.92	2.86	6.0
3.06	1.51	2.64	3.8	3.54	1.77	2.91	5.3
3.08	1.61	2.56	3.8	3.55	1.79	2.79	5.2
3.10	1.54	2.61	4.0	3.57	1.78	3.00	6.2
3.10	1.62	2.62	4.4	3.59	1.89	2.92	5.2
3.10	1.50	2.56	3.8	3.60	1.81	2.95	5.8
3.10	1.61	2.63	3.9	3.60	1.74	3.01	5.9
3.11	1.54	2.63	4.2	3.62	1.83	2.86	5.6
3.11	1.53	2.67	3.8	3.62	1.76	3.03	5.1
3.13	1.47	2.67	4.2	3.62	1.78	3.05	5.9
3.14	1.61	2.56	4.4	3.63	1.84	3.08	5.5
3.15	1.55	2.67	4.3	3.63	1.93	3.07	6.8
3.18	1.61	2.67	4.1	3.64	1.87	2.97	6.6
3.18	1.55	2.70	4.3	3.64	1.85	2.93	6.2
3.19	1.57	2.66	4.3	3.65	1.78	2.98	5.3
3.21	1.65	2.75	5.0	3.65	1.76	3.00	5.9
3.23	1.55	2.76	4.6	3.66	1.91	3.03	5.8
3.24	1.59	2.81	4.8	3.67	1.78	3.00	4.8
3.25	1.60	2.76	4.6	3.70	1.85	3.00	6.4
3.29	1.68	2.84	4.6	3.70	1.80	3.11	6.8
3.29	1.73	2.86	5.3	3.70	1.88	3.11	6.7
3.30	1.64	2.75	4.3	3.70	1.90	3.19	6.7
3.30	1.67	2.74	5.1	3.73	1.90	3.02	5.8
3.31	1.66	2.75	4.3	3.73	1.88	3.13	6.3
3.31	1.58	2.74	4.9	3.73	1.97	3.12	6.1
3.31	1.74	2.88	5.5	3.73	1.98	3.15	6.7
3.32	1.69	2.88	5.1	3.74	1.76	3.06	6.2
3.33	1.65	2.73	4.7	3.75	1.84	3.03	6.3
3.34	1.66	2.84	5.0	3.76	1.91	3.13	7.3
3.34	1.70	2.77	4.8	3.77	1.98	2.97	6.4
3.34	1.65	2.78	5.0	3.79	1.85	3.13	6.6
3.36	1.71	2.80	4.8	3.81	1.93	3.05	6.1
3.36	1.62	2.79	3.9	3.81	1.87	3.10	6.3
3.36	1.70	2.71	4.5	3.81	1.95	3.16	7.5

3.82	1.90	3.15	7 0	4.15	2.08	3.32	8.0
3.83	1.95	3.21	7 7	4.19	2 11	3.39	9.3
3.84	1.94	3.30	7 5	4.20	2 06	3.44	9.4
3.85	1.96	3.40	8 1	4.20	2.16	3.45	8 0
3.86	1.93	3.23	7.4	1 22	2.14	3.50	8.8
3.87	1.96	3.26	6 6	4 30	2 23	3 56	10.3
3.87	1.96	3.35	9 1	4.33	2.10	3 45	9.2
3.87	1.95	3.29	7 5	4.33	2.13	3 55	9.0
3.89	2.02	3 21	6.8	4.38	2 23	3 60	8.7
3.89	1.93	3 22	7.0	4.40	2.15	3 64	8.9
3.92	1.98	3.15	6 8	4.42	2 18	3.70	9 7
3.92	2.04	3 23	6 7	4 43	2.32	3 42	9.8
3.93	1.97	3 16	7 9	4.48	2.20	3 60	8.9
4 00	2.03	3 21	6.9	4.56	2.32	3.60	9.4
4.00	1.98	3 20	6 3	4 60	2 37	3.72	10.2
4 00	2 14	3.17	7 7	4.61	2 33	3 67	11.1
4 08	2.08	3.38	6 8	4 61	2.22	3 73	9.3
4.12	2.09	3.39	9.1	4 73	2 52	3 74	13.1
4 13	2.04	3 36	8 5	4.78	2 50	3.93	12 2
4 15	2	3 49	8 7				

TABLE 5. November 14, 1932.

Length in cm.	Depth in cm.	Height in cm	Weight in gm	Length in cm	Depth in cm	Height in cm	Weight in gm
2.44	1.22	2.10	1 9	3 18	1.60	2.52	4 0
2.50	1.29	2.12	2 5	3.19	1.65	2.58	4 0
2.72	1.39	2.26	2.5	3 20	1.63	2.63	3 8
2.80	1.39	2.35	2.7	3 21	1.52	2 66	4 2
2.80	1.43	2.45	3.0	3.22	1.60	2 62	4 4
2.82	1.29	2.39	2.9	3 23	1.66	2.69	3 7
2.90	1.40	2.48	3.7	3.24	1.63	2 63	4.2
2.92	1.53	2.42	3.4	3 24	1.64	2.69	4.2
2.93	1.45	2.37	3.1	3.24	1.65	2.58	4 1
2.95	1.36	2.46	3.0	3 24	1.71	2.66	4 9
2.95	1.50	2.46	3.1	3.25	1.65	2.66	3 8
2.97	1.41	2.50	3.2	3.26	1.66	2 62	3.8
2.97	1.46	2.51	3.1	3.27	1.61	2 73	4.1
2.97	1.50	2.54	3 7	3.27	1.65	2.70	4.8
2.98	1.47	2.51	3 5	3 30	1.58	2.79	5 0
2.98	1.50	2.53	3.2	3.32	1.62	2.81	4 6
3.00	1.50	2.51	3 7	3 32	1.63	2.79	3 9
3.00	1.51	2.53	3.6	3.32	1.82	2.76	5.0
3.00	1.53	2.47	3.8	3.33	1.62	2.73	4.9
3.02	1.45	2.50	3.8	3.33	1.74	2.83	4.7
3.02	1.59	2.51	3 8	3.36	1.66	2.83	4 4
3.05	1.54	2.49	4.0	3.36	1.70	2 77	4.7
3 07	1.52	2 64	3 9	3.37	1.70	2.80	4.0
3.07	1.56	2.59	4.3	3 37	1.73	2.82	4.5
3.08	1.45	2.56	3.7	3.39	1.76	2.81	4.9
3.08	1.50	2.56	4.2	3.39	1.77	2 84	4.9
3.09	1.56	2.55	3 9	3.40	1.65	2.79	4.5
3.10	1.48	2.50	3.4	3.40	1.70	2.81	5.3
3.12	1.61	2.55	3.9	3.42	1.71	2.83	4.4
3.14	1.57	2.65	4.6	3.43	1.69	2.82	4 7
3.16	1.68	2.62	4.4	3.43	1.70	2.87	4 7
3.17	1.59	2.66	4.5	3.43	1.71	2.81	5.8
3.17	1.64	2.58	3.4	3.43	1.74	2.82	5 6

3.47	1.71	2.83	5.1	3.73	1.88	3.20	6.5
3.48	1.69	2.84	4.8	3.73	1.91	3.06	6.6
3.48	1.71	2.94	4.8	3.74	1.89	3.01	5.6
3.48	1.75	2.90	5.6	3.74	1.90	3.20	6.2
3.48	1.81	2.86	4.9	3.75	1.88	3.18	5.8
3.49	1.79	2.82	5.5	3.78	1.91	3.06	6.6
3.50	1.79	2.90	1.9	3.78	1.98	3.21	7.6
3.51	1.73	2.81	4.5	3.80	1.95	3.23	6.0
3.51	1.73	2.90	4.8	3.85	1.87	3.22	6.5
3.53	1.86	2.95	6.0	3.86	1.91	3.10	6.3
3.56	1.82	2.90	5.4	3.86	2.03	3.30	6.8
3.56	1.93	2.84	5.5	3.87	1.92	3.15	5.7
3.57	1.83	3.00	5.3	3.88	2.00	3.15	8.1
3.58	1.81	2.92	5.8	3.91	2.08	3.19	7.7
3.59	1.75	2.92	5.0	3.92	1.98	3.25	7.0
3.59	1.78	2.89	5.0	3.94	1.98	3.20	7.1
3.59	1.82	2.98	5.3	3.95	1.92	3.21	5.4
3.60	1.78	2.95	5.9	3.95	1.98	3.30	6.8
3.60	1.80	2.97	4.8	3.95	2.04	3.14	7.0
3.60	1.82	2.93	5.8	3.96	1.91	3.24	6.6
3.60	1.87	2.89	5.5	3.97	2.11	3.32	7.2
3.61	1.78	2.95	5.4	3.98	2.03	3.25	7.5
3.61	1.84	3.02	5.7	4.00	2.03	3.22	7.1
3.62	1.74	2.90	5.1	4.03	2.03	3.33	7.5
3.63	1.85	3.00	5.9	4.05	2.12	3.33	7.6
3.63	1.85	3.04	5.7	4.06	2.11	3.17	7.9
3.64	1.81	2.98	5.6	4.12	2.12	3.26	7.9
3.64	1.86	3.07	6.0	4.12	2.13	3.35	8.8
3.64	1.90	2.94	5.4	4.15	2.08	3.53	8.1
3.64	1.92	3.00	6.1	4.16	2.25	3.17	7.2
3.65	1.80	3.11	6.2	4.17	2.14	3.43	8.7
3.67	1.84	3.01	6.5	4.20	2.25	3.38	9.2
3.67	1.90	3.12	6.4	4.22	2.20	3.43	8.0
3.67	1.83	3.00	5.1	4.23	2.10	3.35	7.5
3.68	1.83	3.08	6.0	4.26	2.07	3.49	7.8
3.69	1.83	3.10	5.6	4.36	2.26	3.46	10.7
3.69	1.92	3.05	6.3	4.41	2.22	3.71	9.7
3.71	1.87	3.03	6.6	4.45	2.31	3.53	10.1

TABLE 6. December 14, 1932.

Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Length in cm.	Depth in cm.	Height in cm.	Weight in gm.
2.37	1.19	2.06	2.0	2.71	1.40	2.32	2.7
2.37	1.22	2.08	2.2	2.72	1.31	2.41	2.9
2.41	1.19	2.06	2.1	2.72	1.39	2.30	2.9
2.42	1.17	2.05	1.9	2.74	1.41	2.37	3.0
2.42	1.20	2.06	2.5	2.75	1.37	2.35	2.7
2.54	1.28	2.13	2.2	2.80	1.31	2.36	2.9
2.59	1.26	2.16	2.2	2.80	1.34	2.35	2.6
2.59	1.30	2.29	2.6	2.80	1.40	2.38	2.7
2.61	1.32	2.20	2.5	2.80	1.41	2.45	3.4
2.61	1.37	2.21	2.4	2.80	1.44	2.32	3.8
2.65	1.34	2.26	3.0	2.82	1.39	2.36	3.3
2.66	1.32	2.20	2.1	2.84	1.38	2.40	3.7
2.69	1.31	2.27	2.5	2.84	1.38	2.46	3.2
2.69	1.40	2.31	3.4	2.85	1.37	2.40	3.4
2.71	1.34	2.30	2.6	2.85	1.47	2.52	3.8

Length in cm	Depth in cm	Height in cm	Weight in gm	Length in cm	Depth in cm	Height in cm	Weight in gm.
2.86	1.41	2.40	3.2	3.37	1.75	2.81	4.2
2.86	1.44	2.41	2.9	3.38	1.65	2.87	5.1
2.86	1.44	2.47	3.6	3.39	1.74	2.83	6.1
2.87	1.37	2.36	3.3	3.40	1.70	2.78	4.8
2.88	1.36	2.40	3.0	3.40	1.70	2.81	4.7
2.90	1.40	2.42	3.6	3.40	1.83	2.95	5.9
2.92	1.42	2.48	3.4	3.41	1.66	2.88	5.6
2.93	1.41	2.48	3.0	3.41	1.70	2.83	5.3
2.94	1.45	2.46	2.8	3.43	1.74	2.90	5.2
2.98	1.52	2.61	4.5	3.44	1.64	2.90	6.1
2.98	1.59	2.58	4.0	3.47	1.76	2.86	4.9
3.00	1.48	2.50	4.1	3.48	1.77	2.81	4.9
3.00	1.50	2.49	3.3	3.48	1.80	2.90	5.7
3.01	1.47	2.51	3.6	3.50	1.80	2.84	5.8
3.02	1.52	2.55	4.1	3.53	1.70	2.95	5.0
3.03	1.51	2.54	3.2	3.53	1.86	3.11	6.7
3.03	1.46	2.60	4.2	3.59	1.90	3.01	7.6
3.04	1.50	2.52	3.6	3.59	1.91	2.99	6.3
3.05	1.47	2.53	3.2	3.60	1.82	3.00	6.2
3.07	1.52	2.58	4.6	3.64	1.92	2.97	6.3
3.07	1.57	2.59	1.3	3.65	1.86	2.96	6.0
3.08	1.52	2.59	4.1	3.68	1.83	2.92	5.2
3.09	1.52	2.61	3.8	3.68	1.86	3.11	6.5
3.09	1.53	2.51	3.3	3.69	1.92	3.06	6.6
3.10	1.55	2.63	4.4	3.70	1.90	2.93	6.3
3.10	1.58	2.61	3.8	3.70	1.98	3.00	5.6
3.11	1.57	2.59	4.1	3.73	1.78	2.98	7.1
3.13	1.56	2.58	4.3	3.73	1.93	3.09	7.1
3.13	1.59	2.47	3.7	3.76	1.87	3.03	6.7
3.16	1.51	2.61	3.6	3.76	1.94	3.13	6.5
3.16	1.65	2.59	4.6	3.77	1.87	3.10	5.6
3.19	1.58	2.70	4.3	3.77	1.95	3.15	7.5
3.19	1.60	2.63	1.0	3.78	2.02	3.00	6.6
3.20	1.55	2.68	4.6	3.80	1.84	3.07	6.3
3.20	1.60	2.67	3.9	3.81	1.94	3.12	7.2
3.20	1.60	2.64	5.0	3.82	1.94	3.16	7.9
3.21	1.57	2.73	3.8	3.86	1.96	3.00	6.6
3.21	1.59	2.68	4.6	3.87	1.97	3.11	6.8
3.23	1.58	2.72	5.1	3.87	1.97	3.21	7.1
3.23	1.63	2.64	4.5	3.88	2.00	3.22	8.2
3.23	1.68	2.78	4.6	3.96	1.98	3.27	7.6
3.24	1.67	2.74	4.7	3.96	2.03	3.20	7.6
3.26	1.60	2.67	3.9	3.96	2.03	3.27	7.8
3.26	1.63	2.63	4.1	3.97	2.04	3.19	6.7
3.26	1.65	2.80	5.2	4.02	1.93	3.30	7.9
3.27	1.58	2.75	4.2	4.03	1.92	3.17	6.8
3.29	1.56	2.75	5.1	4.09	2.08	3.27	8.4
3.29	1.60	2.73	4.6	4.10	2.07	3.23	7.4
3.29	1.76	2.82	5.0	4.14	2.10	3.36	8.1
3.30	1.75	2.75	5.1	4.16	2.18	3.51	8.9
3.32	1.56	2.75	4.9	4.17	2.10	3.29	8.1
3.32	1.63	2.76	4.9	4.18	2.08	3.48	8.9
3.33	1.65	2.76	4.6	4.18	2.13	3.49	8.3
3.33	1.69	2.73	4.8	4.20	2.06	3.48	9.4
3.34	1.71	2.87	4.8	4.24	2.16	3.39	7.6
3.34	1.74	2.86	6.6	4.25	1.98	3.33	8.5
3.35	1.65	2.81	4.6	4.28	2.15	3.41	8.6
3.35	1.73	2.83	5.5	4.31	2.10	3.54	8.3
3.35	1.76	2.90	5.8	4.40	2.13	3.55	10.2

4.40	2.23	3.50	10.0	4.67	2.43	3.52	10.3
4.54	2.25	3.62	9.6	4.71	2.38	3.81	14.0
4.57	2.29	3.55	9.3	4.90	2.45	3.86	12.7
4.60	2.38	3.70	10.3	5.02	2.47	3.94	12.3
4.63	2.35	3.58	10.1	5.07	2.65	3.87	14.6
4.63	2.44	3.76	12.7				

TABLE 7. January 14, 1933.

Length in cm.	Depth in cm	Height in cm	Weight in gm	Length in cm.	Depth in cm	Height in cm	Weight in gm
2.42	1.20	2.13	2.1	3.10	1.49	2.51	3.2
2.58	1.31	2.18	2.5	3.10	1.50	2.56	4.0
2.60	1.30	2.17	2.4	3.11	1.48	2.50	4.1
2.60	1.31	2.30	2.8	3.11	1.53	2.65	3.6
2.61	1.30	2.21	3.2	3.11	1.53	2.66	4.2
2.62	1.36	2.17	2.4	3.11	1.55	2.6	3.9
2.63	1.31	2.26	2.3	3.11	1.60	2.60	4.3
2.64	1.35	2.20	2.8	3.11	1.60	2.61	4.1
2.65	1.22	2.29	2.3	3.12	1.46	2.65	1.5
2.70	1.37	2.31	2.6	3.12	1.52	2.52	1.0
2.73	1.40	2.27	3.0	3.12	1.58	2.50	3.6
2.74	1.41	2.33	3.6	3.12	1.58	2.56	4.5
2.75	1.34	2.24	3.2	3.13	1.56	2.70	4.1
2.77	1.37	2.36	3.0	3.14	1.55	2.58	3.4
2.77	1.40	2.39	3.2	3.15	1.62	2.57	3.9
2.77	1.45	2.39	3.2	3.17	1.53	2.61	1.1
2.83	1.40	2.35	3.4	3.20	1.58	2.63	3.7
2.88	1.34	2.44	3.1	3.20	1.60	2.62	4.6
2.88	1.41	2.40	3.1	3.21	1.53	2.66	4.0
2.90	1.36	2.40	3.1	3.23	1.58	2.73	4.2
2.91	1.35	2.47	3.4	3.23	1.59	2.75	4.6
2.91	1.46	2.48	3.1	3.23	1.68	2.66	4.3
2.92	1.44	2.46	2.7	3.24	1.80	2.68	5.0
2.92	1.46	2.44	3.9	3.25	1.60	2.81	5.3
2.93	1.35	2.42	3.0	3.26	1.62	2.67	1.0
2.95	1.43	2.44	3.6	3.26	1.72	2.79	5.6
2.95	1.50	2.46	3.3	3.27	1.65	2.77	5.7
2.95	1.58	2.44	3.9	3.28	1.65	2.67	5.1
2.97	1.45	2.46	3.1	3.29	1.48	2.67	1.2
2.97	1.46	2.49	3.7	3.29	1.63	2.80	1.5
2.97	1.50	2.46	3.2	3.29	1.78	2.81	5.2
2.98	1.49	2.50	3.5	3.30	1.64	2.86	4.4
2.99	1.55	2.50	3.6	3.30	1.70	2.76	5.5
3.00	1.60	2.47	3.6	3.31	1.57	2.72	5.1
3.00	1.63	2.51	4.2	3.32	1.70	2.77	5.2
3.01	1.45	2.50	3.7	3.33	1.60	2.72	3.9
3.01	1.52	2.54	3.7	3.35	1.64	2.84	5.3
3.02	1.49	2.53	3.9	3.35	1.69	2.80	5.1
3.03	1.43	2.55	3.3	3.35	1.71	2.88	5.6
3.03	1.46	2.55	3.3	3.37	1.73	2.81	5.8
3.03	1.54	2.54	3.5	3.38	1.64	2.81	4.9
3.04	1.50	2.58	4.3	3.39	1.71	2.76	1.3
3.05	1.42	2.56	3.4	3.41	1.80	2.83	5.4
3.05	1.47	2.59	3.9	3.42	1.75	2.88	4.8
3.05	1.51	2.68	4.2	3.43	1.85	2.89	5.7
3.06	1.61	2.58	4.1	3.44	1.71	2.81	4.6
3.07	1.60	2.60	4.1	3.45	1.60	2.77	4.1

3.45	1.74	2.83	5.2	3.82	1.98	3.09	6.2
3.46	1.73	2.93	6.5	3.82	2.00	3.08	6.7
3.48	1.79	2.97	5.8	3.83	1.97	3.07	6.3
3.49	1.80	2.88	4.7	3.85	1.95	3.23	7.8
3.50	1.71	2.83	4.5	3.85	1.99	3.17	6.6
3.50	1.80	2.91	5.6	3.85	2.04	3.30	6.9
3.51	1.76	2.91	5.1	3.88	2.00	3.19	8.6
3.52	1.77	2.92	5.0	3.88	2.02	3.21	6.9
3.53	1.71	2.90	5.4	3.88	2.11	3.19	7.5
3.53	1.83	2.91	5.3	3.89	1.92	3.13	6.2
3.55	1.84	2.83	5.3	3.89	1.94	3.09	7.5
3.56	1.68	2.83	4.4	3.89	1.91	3.10	6.9
3.57	1.78	2.88	5.3	3.90	2.15	3.19	7.3
3.58	1.82	2.95	5.7	3.91	1.90	3.11	7.0
3.59	1.85	2.96	5.1	3.91	1.99	3.22	8.1
3.60	1.77	3.01	1.7	3.93	2.00	3.26	6.6
3.60	1.80	2.99	6.1	3.93	2.11	3.23	7.0
3.61	1.82	3.00	5.1	3.95	2.02	3.22	6.8
3.62	1.78	2.87	6.8	3.97	2.02	3.34	7.6
3.62	1.82	2.91	6.0	3.97	2.03	3.25	7.7
3.62	1.88	3.01	5.8	3.99	1.82	3.09	6.0
3.65	1.79	2.93	5.7	4.05	1.97	3.15	7.7
3.65	1.85	2.99	5.7	4.13	2.03	3.25	8.8
3.65	1.87	3.05	7.1	4.15	2.10	3.35	8.2
3.68	1.76	3.08	5.5	4.17	2.20	3.40	9.1
3.68	1.80	3.05	6.7	4.19	2.01	3.36	8.1
3.68	1.85	3.09	5.6	4.20	2.15	3.40	8.3
3.69	1.81	3.07	7.5	4.21	2.10	3.26	7.8
3.69	1.83	2.95	6.5	4.29	2.12	3.50	8.2
3.70	1.88	3.11	6.7	4.32	2.19	3.64	10.0
3.71	1.91	3.10	5.9	4.33	2.28	3.47	9.7
3.72	1.89	3.01	6.3	4.38	2.25	3.54	9.1
3.73	1.99	3.12	6.9	4.50	2.29	3.67	10.2
3.75	1.91	3.04	6.5	4.62	2.34	3.69	10.1
3.77	1.96	3.07	6.8	4.70	2.35	3.67	12.2
3.79	1.90	3.12	5.8	4.88	2.35	3.75	11.2
3.81	1.88	3.05	7.0				

TABLE 8. February 14, 1933.

Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Length in cm.	Depth in cm.	Height in cm.	Weight in gm.
2.68	1.26	2.26	2.5	2.98	1.39	2.46	3.3
2.71	1.39	2.28	3.0	2.98	1.48	2.54	3.4
2.75	1.41	2.34	3.1	2.98	1.50	2.65	3.5
2.81	1.53	2.35	3.2	3.00	1.47	2.50	3.9
2.85	1.42	2.39	3.2	3.00	1.52	2.58	3.1
2.87	1.50	2.46	3.9	3.01	1.51	2.47	3.8
2.89	1.41	2.42	3.1	3.01	1.51	2.53	3.4
2.89	1.43	2.47	3.3	3.01	1.56	2.50	3.4
2.89	1.46	2.51	3.4	3.03	1.58	2.63	4.3
2.92	1.43	2.38	3.3	3.04	1.46	2.46	3.8
2.92	1.44	2.50	3.9	3.04	1.50	2.51	3.2
2.92	1.45	2.39	3.6	3.06	1.47	2.60	3.9
2.92	1.45	2.45	3.4	3.07	1.58	2.62	4.3
2.93	1.45	2.50	3.4	3.08	1.50	2.48	4.0
2.97	1.41	2.48	3.6	3.08	1.61	2.73	4.7
2.97	1.50	2.48	3.8	3.09	1.58	2.63	4.5

Length in cm	Depth in cm	Height in cm	Weight in gm	Length in cm.	Depth in cm	Height in cm	Weight in gm
3.10	1.53	2.55	3.8	3.48	1.71	2.88	5.2
3.10	1.55	2.58	4.0	3.48	1.76	2.96	5.3
3.10	1.53	2.60	4.4	3.48	1.71	2.94	5.3
3.10	1.55	2.65	4.1	3.49	1.81	2.96	6.1
3.11	1.50	2.68	4.2	3.49	1.66	2.83	4.9
3.11	1.61	2.58	4.4	3.49	1.77	2.92	5.2
3.11	1.55	2.60	4.8	3.51	1.91	3.11	6.3
3.12	1.54	2.64	3.9	3.52	1.70	2.81	5.0
3.12	1.57	2.63	4.4	3.52	1.68	2.85	5.4
3.14	1.54	2.65	4.5	3.53	1.71	2.90	5.6
3.15	1.61	2.61	3.8	3.53	1.79	2.91	5.8
3.16	1.60	2.73	4.4	3.53	1.81	2.98	6.2
3.16	1.61	2.68	3.9	3.54	1.80	2.85	5.4
3.18	1.55	2.54	3.9	3.55	1.78	2.96	5.7
3.18	1.66	2.71	4.5	3.58	1.80	2.95	6.6
3.20	1.52	2.70	3.8	3.58	1.78	2.93	5.5
3.20	1.64	2.68	4.3	3.59	1.88	2.97	6.1
3.20	1.71	2.69	3.6	3.59	1.78	2.89	5.4
3.21	1.65	2.65	1.6	3.59	1.80	2.94	5.7
3.23	1.55	2.79	5.4	3.60	1.78	2.88	5.8
3.25	1.67	2.67	4.1	3.60	1.86	2.94	6.1
3.26	1.56	2.73	4.7	3.60	1.86	3.01	6.2
3.26	1.63	2.81	5.1	3.61	1.76	2.96	5.7
3.27	1.68	2.72	5.3	3.62	1.74	3.01	5.5
3.27	1.69	2.83	5.6	3.62	1.84	3.07	5.2
3.28	1.61	2.73	5.1	3.62	1.79	2.91	6.2
3.28	1.65	2.80	4.7	3.62	1.83	2.93	6.1
3.29	1.75	2.72	5.3	3.62	1.88	3.05	6.6
3.30	1.64	2.78	4.8	3.62	1.92	2.99	6.5
3.30	1.68	2.67	4.6	3.63	1.75	2.98	5.6
3.30	1.70	2.75	4.5	3.63	1.82	3.03	6.3
3.31	1.62	2.70	4.3	3.65	1.83	2.91	5.9
3.31	1.74	2.73	4.4	3.67	1.88	2.99	6.5
3.32	1.65	2.74	4.2	3.68	1.78	2.93	5.7
3.32	1.63	2.75	4.6	3.68	1.91	3.00	7.1
3.32	1.61	2.80	5.1	3.70	1.83	3.00	6.0
3.33	1.65	2.70	4.3	3.70	1.92	3.04	6.4
3.33	1.66	2.83	5.1	3.70	1.74	3.10	5.8
3.33	1.75	2.76	4.7	3.70	1.90	3.07	6.6
3.34	1.64	2.78	4.8	3.70	1.89	3.05	6.1
3.34	1.66	2.79	4.8	3.70	1.91	3.00	7.8
3.35	1.75	2.78	4.8	3.72	1.83	2.96	5.8
3.37	1.67	2.72	4.7	3.72	1.85	2.99	6.3
3.37	1.72	2.80	5.5	3.72	1.89	3.13	7.2
3.38	1.66	2.70	4.6	3.73	1.95	3.04	6.5
3.38	1.66	2.71	3.9	3.76	1.86	3.03	6.1
3.38	1.70	2.80	5.2	3.78	1.85	3.10	7.3
3.39	1.58	2.80	5.5	3.80	1.85	3.13	6.7
3.39	1.76	2.80	5.6	3.85	1.86	3.10	6.5
3.43	1.60	2.74	4.9	3.86	2.00	3.11	5.5
3.43	1.74	2.85	5.5	3.88	1.96	3.06	8.1
3.44	1.62	2.81	5.0	3.89	2.14	3.28	8.8
3.44	1.71	2.80	5.3	3.92	1.95	3.11	6.7
3.44	1.70	2.87	4.9	3.92	1.97	3.23	7.6
3.45	1.80	2.92	5.8	3.93	2.05	3.22	7.6
3.45	1.85	2.88	5.0	3.93	2.11	3.19	8.6
3.47	1.75	2.83	5.0	4.01	1.92	3.18	6.7
3.47	1.78	2.92	5.6	4.01	2.09	3.30	8.7
3.47	1.85	2.90	5.8	4.05	2.08	3.43	7.3

4.09	2.01	3.26	6.7	4.56	2.23	3.66	9.6
4.10	2.15	3.31	7.8	4.71	2.31	3.60	9.9
4.20	2.11	3.30	7.9	4.97	2.44	4.10	14.0
4.41	2.30	3.66	11.5	5.05	2.50	3.99	14.5
4.53	2.33	3.68	11.3				

TABLE 9. March 14, 1933.

Length in cm	Depth in cm	Height in cm	Weight in gm	Length in cm	Depth in cm	Height in cm	Weight in gm
2.87	1.47	2.36	3.5	3.37	1.73	2.85	5.0
2.89	1.44	2.41	3.3	3.38	1.70	2.72	4.1
2.90	1.50	2.50	3.4	3.38	1.77	2.69	4.5
2.97	1.48	2.52	4.2	3.39	1.68	2.74	5.4
2.98	1.58	2.54	3.9	3.39	1.69	2.78	4.9
3.00	1.46	2.44	3.5	3.39	1.72	2.83	5.0
3.05	1.55	2.62	4.7	3.39	1.74	2.72	3.7
3.06	1.50	2.52	3.2	3.39	1.76	2.75	4.7
3.06	1.53	2.53	3.3	3.40	1.65	2.74	3.8
3.06	1.61	2.76	4.7	3.40	1.73	2.83	4.9
3.10	1.57	2.56	3.4	3.42	1.72	2.82	4.9
3.11	1.55	2.66	4.5	3.45	1.68	2.89	4.8
3.12	1.52	2.64	4.1	3.45	1.80	2.82	5.8
3.12	1.59	2.63	3.6	3.46	1.69	2.93	5.3
3.13	1.48	2.52	3.8	3.46	1.74	2.87	5.2
3.15	1.60	2.67	4.6	3.48	1.66	2.83	4.3
3.16	1.60	2.59	4.2	3.48	1.73	2.88	5.0
3.18	1.71	2.73	5.4	3.49	1.68	2.79	5.2
3.19	1.66	2.51	4.1	3.49	1.69	2.96	4.6
3.19	1.65	2.77	5.1	3.49	1.74	2.91	5.3
3.19	1.67	2.65	3.7	3.50	1.73	2.90	5.0
3.21	1.69	2.77	4.6	3.50	1.77	2.87	5.2
3.22	1.70	2.69	5.0	3.51	1.73	2.82	5.4
3.23	1.60	2.61	4.4	3.51	1.75	2.84	5.7
3.23	1.59	2.70	3.9	3.51	1.85	2.96	5.9
3.24	1.56	2.79	1.5	3.53	1.77	2.84	4.7
3.24	1.67	2.67	5.1	3.53	1.84	2.95	6.3
3.26	1.61	2.65	3.8	3.53	1.89	2.97	5.7
3.26	1.61	2.67	3.9	3.54	1.67	2.95	6.6
3.26	1.62	2.61	4.3	3.54	1.70	2.86	4.6
3.28	1.65	2.77	5.7	3.54	1.75	2.86	4.6
3.28	1.65	2.78	4.8	3.54	1.76	2.87	5.3
3.28	1.67	2.73	4.6	3.54	1.81	3.00	5.3
3.29	1.65	2.80	4.9	3.54	1.84	2.96	5.7
3.29	1.70	2.81	4.7	3.57	1.76	2.85	4.9
3.30	1.64	2.83	4.2	3.58	1.79	2.89	5.4
3.30	1.68	2.73	4.6	3.58	1.80	2.93	6.0
3.30	1.68	2.76	4.5	3.58	1.80	2.95	5.9
3.30	1.71	2.73	4.3	3.58	1.80	2.97	5.2
3.31	1.59	2.71	5.0	3.59	1.80	2.90	5.4
3.31	1.70	2.72	3.9	3.60	1.72	3.01	5.6
3.33	1.69	2.81	5.2	3.60	1.80	2.94	6.7
3.34	1.68	2.77	4.8	3.60	1.81	2.91	5.8
3.34	1.69	2.74	5.0	3.61	1.75	2.90	5.2
3.35	1.65	2.70	4.3	3.61	1.77	3.00	5.1
3.35	1.67	2.67	4.9	3.62	1.77	2.96	5.6
3.36	1.68	2.72	4.9	3.62	1.90	3.07	7.1
3.37	1.67	2.77	4.7	3.62	1.77	2.90	5.0

3.63	1.77	2.90	5.4	3.83	1.97	3.00	6.3
3.63	1.71	2.99	5.4	3.85	1.86	3.05	6.0
3.63	1.78	2.91	5.3	3.85	1.97	3.23	7.3
3.63	1.86	2.76	5.6	3.85	1.91	3.09	6.5
3.65	1.84	2.97	5.7	3.86	2.03	3.22	7.8
3.67	1.85	3.01	5.8	3.87	1.83	3.07	6.2
3.68	1.76	2.92	5.6	3.90	1.90	3.11	6.7
3.68	1.76	2.93	5.4	3.90	1.95	3.19	7.0
3.70	1.86	3.03	6.1	3.90	1.97	3.09	6.9
3.70	1.87	2.99	6.3	3.91	1.91	3.23	7.0
3.70	1.91	2.97	5.3	3.92	1.90	3.25	6.6
3.70	1.95	3.01	6.8	3.94	1.92	3.21	6.8
3.71	1.96	3.08	6.2	4.01	2.04	3.28	8.1
3.73	1.82	3.02	6.1	4.02	1.91	3.25	6.7
3.73	1.88	3.03	6.4	4.04	2.03	3.38	7.7
3.73	1.98	2.91	6.9	4.12	2.17	3.38	7.0
3.74	1.90	3.08	7.1	4.18	2.16	3.45	8.9
3.75	1.84	3.06	5.6	4.21	2.28	3.48	8.6
3.78	1.94	3.13	6.8	4.24	2.08	3.40	8.8
3.78	1.97	3.09	7.0	4.29	2.13	3.49	8.9
3.79	1.89	3.20	7.4	4.53	2.25	3.55	9.9
3.80	1.82	3.13	6.1	4.72	2.40	3.93	11.0
3.80	2.05	3.20	8.0	4.78	2.48	3.75	12.4
3.81	1.86	3.11	7.3	4.84	2.40	3.81	12.3
3.81	1.93	3.05	7.0	4.93	2.43	4.05	13.0

TABLE 10. April 14, 1933

Length in cm.	Depth in cm.	Height in cm.	Weight in gm.	Length in cm.	Depth in cm.	Height in cm.	Weight in gm.
2.70	1.37	2.32	2.2	3.15	1.54	2.58	4.3
2.80	1.43	2.34	3.7	3.15	1.58	2.60	4.4
2.86	1.41	2.39	3.7	3.16	1.60	2.62	4.8
2.87	1.38	2.35	2.9	3.17	1.60	2.60	4.5
2.87	1.40	2.39	3.0	3.18	1.53	2.71	4.7
2.88	1.46	2.34	3.9	3.18	1.64	2.61	4.2
2.88	1.45	2.44	2.9	3.19	1.56	2.62	4.2
2.90	1.48	2.40	3.8	3.20	1.60	2.62	3.5
2.91	1.46	2.44	4.1	3.20	1.63	2.70	1.1
2.92	1.52	2.48	3.9	3.20	1.56	2.71	1.2
2.95	1.55	2.48	4.2	3.22	1.70	2.67	4.2
2.96	1.50	2.45	3.5	3.24	1.68	2.72	5.0
2.97	1.42	2.50	3.5	3.24	1.60	2.79	5.1
2.97	1.49	2.40	3.8	3.25	1.60	2.71	1.4
2.99	1.46	2.41	4.1	3.25	1.65	2.60	4.6
3.00	1.56	2.46	4.0	3.25	1.72	2.69	4.6
3.03	1.57	2.63	3.9	3.26	1.59	2.78	5.3
3.04	1.51	2.54	3.2	3.26	1.75	2.76	4.8
3.06	1.52	2.62	3.6	3.26	1.63	2.69	3.7
3.06	1.63	2.60	3.6	3.27	1.72	2.77	5.0
3.08	1.50	2.66	4.2	3.27	1.58	2.79	4.3
3.08	1.69	2.61	4.9	3.27	1.70	2.64	5.3
3.10	1.58	2.59	4.5	3.28	1.61	2.72	4.3
3.11	1.57	2.57	4.2	3.28	1.69	2.77	4.8
3.11	1.48	2.63	3.6	3.29	1.60	2.69	4.0
3.11	1.50	2.52	3.2	3.29	1.71	2.81	4.8
3.14	1.65	2.67	4.4	3.29	1.72	2.85	5.1
3.15	1.64	2.61	4.1	3.30	1.70	2.73	4.7

3.31	1.67	2.73	1.7	3.68	1.90	3.10	5.3
3.31	1.71	2.66	4.4	3.68	1.90	3.04	6.7
3.34	1.64	2.71	1.1	3.68	1.82	3.04	6.5
3.34	1.80	2.78	4.5	3.68	1.81	3.05	6.0
3.34	1.63	2.76	5.7	3.68	1.86	3.05	4.9
3.34	1.72	2.72	4.7	3.69	1.85	3.05	6.1
3.35	1.68	2.78	4.8	3.70	1.86	3.08	6.7
3.37	1.71	2.78	4.7	3.70	1.87	3.05	5.7
3.38	1.74	2.77	4.6	3.70	1.95	2.95	6.0
3.38	1.70	2.82	4.9	3.70	1.95	3.10	6.5
3.38	1.62	2.77	4.2	3.71	1.82	3.02	5.6
3.38	1.70	2.73	4.8	3.72	1.83	3.08	7.3
3.40	1.77	2.88	4.6	3.73	1.87	3.00	6.5
3.40	1.76	2.81	4.7	3.73	1.86	3.14	7.2
3.41	1.80	2.76	4.8	3.73	1.94	3.07	6.3
3.42	1.79	2.92	5.2	3.75	1.90	3.05	6.2
3.43	1.63	2.83	4.7	3.75	1.89	2.99	6.2
3.43	1.72	2.85	5.2	3.77	1.84	3.14	6.8
3.45	1.77	2.86	5.4	3.77	2.03	3.10	6.8
3.46	1.74	2.80	5.1	3.77	1.89	2.99	6.3
3.46	1.68	2.87	4.9	3.80	1.92	3.17	7.1
3.47	1.77	2.83	5.3	3.82	1.89	3.17	8.1
3.47	1.84	2.92	4.7	3.83	1.86	3.06	5.9
3.48	1.72	2.85	5.4	3.85	1.90	3.14	6.7
3.48	1.80	2.85	6.1	3.89	1.99	3.26	7.4
3.50	1.80	2.95	5.7	3.90	2.01	3.22	7.3
3.50	1.80	2.03	6.4	3.91	1.99	3.20	7.2
3.50	1.84	2.82	5.7	3.92	1.99	3.17	7.8
3.50	1.76	2.82	4.9	3.92	2.05	3.31	8.4
3.51	1.78	2.83	5.4	3.96	1.93	3.16	7.6
3.54	1.77	2.80	5.1	3.97	2.05	3.23	8.4
3.54	1.76	2.95	5.6	3.97	2.11	3.32	7.5
3.55	1.78	2.87	6.0	3.98	2.21	3.19	9.0
3.55	1.88	2.97	5.4	4.06	2.01	3.42	8.6
3.56	1.87	2.90	5.3	4.06	2.09	3.34	8.4
3.58	1.79	2.93	4.9	4.08	2.02	3.42	8.2
3.60	1.84	2.96	6.0	4.11	2.05	3.39	8.5
3.61	1.78	2.98	5.7	4.17	2.10	3.22	7.7
3.61	1.88	2.88	6.3	4.28	2.21	3.53	10.3
3.63	1.88	3.02	5.6	4.30	2.08	3.44	9.9
3.64	1.92	3.08	6.6	4.30	2.21	3.54	8.1
3.66	1.91	2.92	5.8	4.36	2.18	3.47	7.8
3.67	1.77	2.85	5.7	4.48	2.13	3.59	9.5
3.67	1.91	3.08	6.4	4.53	2.31	3.57	10.9
3.67	1.90	2.95	5.6				

TABLE 11. May 14, 1933.

Length in cm	Depth in cm.	Height in cm	Weight in gm.	Length in cm	Depth in cm	Height in cm.	Weight in gm.
2.71	1.30	2.34	2.2	2.97	1.57	2.48	4.1
2.75	1.32	2.27	2.9	2.98	1.53	2.45	3.8
2.83	1.41	2.33	3.0	3.00	1.60	2.52	3.6
2.89	1.48	2.31	3.6	3.01	1.50	2.59	3.5
2.89	1.51	2.45	3.3	3.03	1.63	2.56	3.9
2.90	1.40	2.42	3.3	3.05	1.50	2.51	3.8
2.94	1.54	2.45	3.1	3.06	1.51	2.67	4.5
2.94	1.57	2.40	3.4	3.06	1.55	2.63	4.3

Length in cm	Depth in cm.	Height in cm	Weight in gm	Length in cm	Depth in cm	Height in cm	Weight in gm
3.07	1.58	2.50	3.5	3.60	1.89	3.00	6.1
3.09	1.68	2.44	4.2	3.61	1.81	2.93	1.7
3.10	1.60	2.64	3.5	3.61	1.86	2.92	6.3
3.15	1.55	2.61	3.8	3.62	1.81	2.95	5.3
3.15	1.58	2.60	3.7	3.63	1.80	2.96	5.0
3.15	1.60	2.58	1.0	3.64	1.77	2.85	1.8
3.20	1.62	2.59	4.6	3.65	1.92	2.93	5.2
3.22	1.60	2.61	4.6	3.67	1.81	3.15	6.5
3.23	1.62	2.73	4.1	3.67	1.93	3.03	5.0
3.24	1.61	2.77	3.9	3.68	1.93	3.06	5.2
3.24	1.65	2.72	4.2	3.69	1.81	2.94	5.1
3.24	1.71	2.73	4.3	3.71	1.90	3.19	7.3
3.25	1.65	2.75	3.6	3.73	1.85	3.06	6.2
3.25	1.68	2.69	4.8	3.73	1.91	3.06	5.0
3.26	1.65	2.67	4.6	3.75	2.06	3.00	5.9
3.27	1.60	2.68	4.1	3.76	1.80	3.10	5.3
3.27	1.71	2.72	4.5	3.77	1.85	2.95	5.1
3.27	1.77	2.72	4.8	3.77	1.86	3.11	5.9
3.29	1.58	2.73	4.8	3.77	1.93	2.95	6.4
3.29	1.70	2.78	4.2	3.77	1.95	3.17	6.4
3.30	1.70	2.76	5.0	3.78	1.81	3.05	6.3
3.31	1.60	2.71	4.9	3.78	1.92	3.20	7.1
3.31	1.68	2.67	4.1	3.79	1.91	3.18	7.4
3.31	1.70	2.70	4.5	3.79	1.99	3.19	7.0
3.31	1.70	2.73	4.4	3.79	2.02	3.01	7.1
3.32	1.64	2.90	5.7	3.80	1.86	3.13	7.5
3.34	1.70	2.72	4.4	3.80	1.95	3.09	5.4
3.35	1.70	2.86	4.6	3.83	2.08	3.26	7.2
3.35	1.75	2.80	4.9	3.84	1.85	3.19	7.0
3.38	1.63	2.85	5.1	3.87	2.05	3.20	8.2
3.38	1.74	2.78	4.8	3.89	1.98	3.23	7.3
3.40	1.71	2.83	5.7	3.90	1.89	3.17	7.0
3.40	1.73	2.75	4.7	3.91	2.08	3.15	7.8
3.40	1.75	2.81	5.4	3.92	1.97	3.16	7.2
3.41	1.70	2.78	4.7	3.93	2.11	3.23	8.0
3.41	1.74	2.74	5.0	3.95	1.98	3.19	7.0
3.41	1.78	2.89	4.4	3.97	2.04	3.13	7.0
3.43	1.70	2.90	5.6	4.00	2.04	3.26	7.8
3.43	1.77	2.83	4.8	4.01	2.07	3.28	7.6
3.44	1.66	2.80	5.0	4.05	1.91	3.31	7.4
3.46	1.81	2.84	5.3	4.07	2.01	3.37	8.3
3.47	1.72	2.96	6.5	4.09	2.07	3.26	9.0
3.48	1.81	2.83	5.3	4.11	2.13	3.39	8.8
3.49	1.71	2.88	4.1	4.12	2.07	3.35	8.2
3.49	1.78	2.88	5.7	4.14	2.05	3.41	7.9
3.51	1.75	2.88	5.3	4.15	2.08	3.18	7.3
3.52	1.72	2.88	4.9	4.16	2.02	3.31	7.1
3.52	1.78	2.87	5.5	4.23	1.97	3.43	7.7
3.53	1.69	2.85	5.3	4.23	2.06	3.33	8.3
3.55	1.81	2.86	4.7	4.25	2.17	3.47	9.7
3.57	1.91	3.01	6.0	4.30	2.12	3.49	9.4
3.58	1.74	3.02	6.6	4.31	2.21	3.24	7.9
3.58	1.81	3.03	5.9	4.38	2.22	3.57	10.8
3.59	1.77	2.94	5.9	4.54	2.23	3.73	10.4
3.59	1.82	3.00	5.5	4.71	2.43	3.68	11.6
3.59	1.92	2.81	5.6	4.75	2.48	3.80	11.5
3.60	1.75	2.91	5.3	4.79	2.46	3.67	11.4
3.60	1.80	3.09	5.8	5.03	2.82	4.07	16.1
3.60	1.85	2.91	7.1	5.04	2.72	3.94	17.3

TABLE 12. June 14, 1933

Length in cm	Depth in cm	Height in cm	Weight in gm	Length in cm.	Depth in cm	Height in cm	Weight in gm
3.00	1.50	2.51	3.6	3.57	1.90	2.83	6.2
3.05	1.52	2.50	3.4	3.57	1.85	3.06	6.1
3.06	1.51	2.60	3.9	3.58	1.86	2.87	5.3
3.07	1.52	2.60	3.8	3.59	1.90	2.93	6.3
3.08	1.51	2.55	3.4	3.60	1.83	2.95	5.8
3.09	1.59	2.60	4.1	3.60	1.77	3.11	6.1
3.11	1.53	2.53	3.4	3.61	1.89	3.00	5.7
3.11	1.60	2.60	3.4	3.62	1.86	3.02	5.9
3.13	1.59	2.65	4.0	3.64	1.85	3.00	5.8
3.14	1.55	2.63	4.0	3.65	1.81	3.05	6.1
3.15	1.60	2.61	4.4	3.66	1.87	3.05	7.0
3.16	1.65	2.64	4.1	3.66	1.90	3.09	6.0
3.21	1.62	2.72	4.7	3.68	1.84	3.00	6.3
3.21	1.67	2.63	4.3	3.70	1.91	3.12	6.2
3.21	1.57	2.74	4.8	3.70	1.72	3.04	5.6
3.21	1.58	2.72	4.2	3.71	1.84	3.12	5.6
3.22	1.62	2.65	3.8	3.71	1.89	3.14	6.7
3.24	1.57	2.70	4.5	3.71	1.81	3.01	6.4
3.24	1.70	2.71	4.1	3.72	1.73	2.93	5.7
3.26	1.62	2.72	4.9	3.74	1.83	3.01	5.7
3.26	1.63	2.74	5.0	3.75	1.90	3.09	6.3
3.33	1.70	2.72	5.1	3.77	1.80	2.14	7.1
3.35	1.73	2.79	4.8	3.77	1.92	3.13	6.2
3.36	1.70	2.87	4.3	3.78	1.89	3.16	5.9
3.38	1.60	2.86	5.4	3.78	1.87	3.14	6.7
3.39	1.66	2.82	5.3	3.78	1.90	3.23	6.7
3.40	1.68	2.86	5.6	3.79	1.91	3.10	6.5
3.40	1.82	2.91	5.7	3.81	1.85	3.15	7.1
3.41	1.70	2.76	4.6	3.81	1.95	3.17	6.2
3.45	1.70	2.81	4.5	3.82	1.94	3.11	7.7
3.45	1.73	2.98	5.7	3.83	1.94	3.22	6.9
3.46	1.78	2.91	5.3	3.84	1.91	3.15	6.2
3.46	1.73	2.84	5.2	3.86	1.95	3.10	6.3
3.46	1.76	2.88	4.9	3.87	1.96	3.19	6.8
3.47	1.76	2.88	5.4	3.90	1.91	3.21	6.4
3.47	1.84	2.91	5.1	3.90	1.94	3.30	6.6
3.48	1.77	2.87	5.5	3.92	2.01	3.37	7.5
3.49	1.77	2.90	5.9	3.93	1.92	3.12	6.8
3.49	1.76	3.00	5.5	3.96	1.93	3.32	8.5
3.51	1.73	2.94	4.6	3.96	2.02	3.12	7.2
3.52	1.75	2.93	5.5	3.96	2.02	3.26	7.7
3.53	1.83	2.95	5.2	4.00	1.92	3.40	8.5
3.53	1.76	3.09	6.0	4.00	1.96	3.23	8.5
3.55	1.84	3.08	5.1	4.08	2.02	3.40	7.9
3.55	1.70	3.00	6.4	4.09	2.08	3.33	7.7

TABLE 13. July 14, 1933.

Length in cm.	Depth in cm	Height in cm	Weight in gm.	Length in cm	Depth in cm.	Height in cm.	Weight in gm
2.60	1.28	2.20	2.3	2.87	1.42	2.51	3.8
2.61	1.27	2.25	2.7	2.92	1.38	2.44	2.7
2.78	1.41	2.36	3.0	2.99	1.40	2.48	2.9

Length in cm	Depth in cm	Height in cm.	Weight in gm.	Length in cm.	Depth in cm	Height in cm	Weight in gm
3.01	1.50	2.60	3.5	3.60	1.86	2.96	6.6
3.05	1.59	2.63	4.4	3.60	1.86	2.93	5.9
3.07	1.40	2.52	3.7	3.60	1.86	3.12	6.9
3.08	1.58	2.62	4.0	3.61	1.73	3.08	6.7
3.11	1.60	2.57	3.7	3.61	1.82	2.91	5.1
3.16	1.62	2.69	4.3	3.62	1.74	3.01	6.5
3.17	1.61	2.74	5.0	3.62	1.75	3.09	7.0
3.20	1.58	2.75	4.9	3.62	1.77	3.00	6.7
3.21	1.61	2.75	4.8	3.63	1.81	3.03	7.0
3.21	1.67	2.74	4.2	3.64	1.79	3.06	6.7
3.21	1.67	2.75	4.7	3.64	1.82	3.01	6.7
3.22	1.60	2.71	5.3	3.64	1.72	3.03	6.0
3.24	1.61	2.68	4.7	3.65	1.81	3.09	7.0
3.27	1.63	2.78	5.0	3.65	1.86	3.03	6.5
3.28	1.70	2.76	5.7	3.65	1.95	3.10	6.3
3.29	1.67	2.80	5.1	3.66	1.77	3.06	6.6
3.29	1.70	2.89	5.3	3.66	1.87	3.10	7.4
3.30	1.62	2.70	5.3	3.66	1.90	3.11	6.6
3.32	1.66	2.76	4.4	3.67	1.93	3.09	6.4
3.33	1.64	2.86	5.3	3.68	1.88	3.05	7.1
3.33	1.68	2.76	5.5	3.68	1.88	3.16	8.4
3.35	1.70	2.81	4.5	3.69	1.79	3.16	7.3
3.36	1.66	2.76	5.4	3.70	1.84	3.01	5.8
3.38	1.75	2.97	6.2	3.71	1.89	3.23	7.7
3.38	1.79	2.78	5.3	3.72	1.86	3.05	6.3
3.39	1.68	2.86	5.1	3.73	1.80	3.15	7.1
3.39	1.74	2.88	5.5	3.74	1.97	3.15	7.3
3.40	1.65	2.77	4.7	3.76	1.79	3.20	7.5
3.40	1.72	2.84	4.9	3.77	1.90	3.11	7.1
3.40	1.75	2.86	5.6	3.77	1.91	3.10	7.3
3.41	1.78	3.03	7.0	3.82	1.90	3.14	8.2
3.42	1.67	2.99	6.2	3.83	1.80	3.23	7.4
3.42	1.70	2.97	6.2	3.86	1.89	3.20	7.5
3.45	1.62	2.89	5.5	3.87	1.90	3.20	6.9
3.45	1.64	2.85	4.8	3.87	1.92	3.32	8.3
3.45	1.78	3.02	6.0	3.88	1.85	3.24	6.4
3.47	1.67	3.00	5.9	3.88	1.99	3.14	7.9
3.47	1.72	2.91	6.4	3.93	1.90	3.33	8.0
3.48	1.73	2.91	5.7	3.95	1.91	3.29	8.0
3.48	1.74	2.94	6.3	3.96	1.89	3.23	7.5
3.49	1.80	2.96	6.1	3.97	1.97	3.31	9.3
3.50	1.73	2.96	5.1	3.99	1.93	3.33	9.0
3.52	1.87	2.95	5.0	3.99	1.95	3.30	8.3
3.53	1.71	2.92	4.6	4.00	1.92	3.33	8.0
3.53	1.75	2.90	5.2	4.00	2.00	3.38	9.5
3.53	1.81	3.00	5.9	4.05	2.01	3.40	8.5
3.55	1.73	3.10	6.8	4.05	2.08	3.14	9.1
3.56	1.77	3.02	6.3	4.07	1.90	3.36	7.8
3.56	1.81	2.96	6.1	4.13	2.04	3.47	9.3
3.56	1.85	3.02	6.6	4.16	2.15	3.54	9.1
3.57	1.77	3.01	6.1	4.16	1.95	3.40	8.6
3.58	1.91	3.05	7.5	4.17	2.12	3.24	8.3
3.59	1.78	3.05	6.6	4.23	2.05	3.49	9.6
3.60	1.74	3.03	5.8	4.29	2.14	3.47	9.7
3.60	1.82	3.14	6.6	4.44	2.25	3.55	10.6

TABLE 14. August 14, 1933.

Length in cm	Depth in cm	Height in cm	Weight in gm	Length in cm.	Depth in cm.	Height in cm	Weight in gm
2.66	1.31	2.20	2.2	3.50	1.82	2.84	4.9
2.67	1.34	2.20	2.6	3.52	1.81	2.90	5.2
2.74	1.27	2.25	1.9	3.53	1.83	2.85	6.0
2.89	1.46	2.46	3.1	3.53	1.85	3.00	5.5
2.95	1.47	2.53	4.1	3.54	1.83	2.96	5.8
3.00	1.50	2.40	3.8	3.55	1.86	2.98	5.8
3.02	1.49	2.50	4.0	3.56	1.96	2.96	5.8
3.03	1.60	2.53	3.4	3.57	1.77	2.91	5.5
3.04	1.56	2.53	3.4	3.57	1.83	3.00	5.3
3.11	1.53	2.56	3.6	3.58	1.83	3.03	6.2
3.13	1.58	2.61	3.3	3.59	1.73	2.87	5.4
3.14	1.58	2.57	4.0	3.60	1.81	3.05	6.3
3.15	1.56	2.56	3.5	3.60	1.83	2.92	5.0
3.18	1.57	2.59	4.3	3.62	1.76	2.87	4.8
3.18	1.66	2.69	5.1	3.62	1.80	2.87	5.5
3.19	1.53	2.63	4.8	3.63	1.83	2.97	4.8
3.20	1.58	2.70	4.7	3.63	1.84	3.12	7.1
3.20	1.59	2.63	3.7	3.63	1.85	2.98	5.7
3.20	1.69	2.73	5.0	3.65	1.84	3.08	6.3
3.21	1.66	2.80	4.7	3.67	1.79	3.03	6.4
3.21	1.73	2.73	4.6	3.67	1.80	2.99	5.5
3.22	1.56	2.67	4.0	3.67	1.93	2.99	6.4
3.25	1.64	2.78	5.3	3.68	1.85	3.15	5.6
3.26	1.60	2.76	5.1	3.69	1.87	3.03	5.5
3.28	1.75	2.71	4.3	3.70	1.87	3.11	6.3
3.29	1.65	2.77	4.5	3.71	1.94	2.97	5.5
3.31	1.63	2.73	3.9	3.71	1.99	3.15	6.1
3.33	1.63	2.79	4.8	3.72	1.80	2.95	5.7
3.33	1.64	2.81	4.2	3.72	1.90	3.07	6.1
3.34	1.73	2.77	4.6	3.73	1.82	3.11	5.7
3.36	1.59	2.77	4.1	3.73	1.87	3.13	7.2
3.37	1.69	2.71	4.2	3.73	1.93	3.11	5.7
3.37	1.73	2.88	4.8	3.75	1.90	3.01	5.8
3.37	1.74	2.81	4.3	3.75	1.95	3.10	6.6
3.39	1.69	2.86	4.5	3.76	1.82	3.10	5.9
3.39	1.72	2.90	5.6	3.78	1.85	3.09	5.7
3.40	1.73	2.80	5.0	3.78	1.91	3.00	6.8
3.40	1.78	2.86	5.7	3.79	1.81	3.03	5.5
3.41	1.63	2.76	5.0	3.80	1.87	3.10	5.5
3.41	1.79	2.94	4.9	3.80	2.01	3.14	7.8
3.42	1.66	2.80	5.4	3.81	1.82	3.14	6.1
3.42	1.71	2.82	4.7	3.81	1.85	3.10	7.3
3.42	1.72	2.78	5.0	3.85	1.88	3.06	5.8
3.43	1.72	2.70	4.9	3.85	2.03	3.20	7.1
3.45	1.71	2.75	5.1	3.87	1.94	3.16	7.1
3.45	1.71	2.90	5.0	3.87	1.96	3.19	6.7
3.45	1.75	2.96	5.5	3.87	2.00	3.12	7.7
3.45	1.84	2.92	6.3	3.88	1.90	3.15	6.2
3.47	1.78	2.75	4.7	3.88	1.91	3.07	5.5
3.48	1.75	2.87	5.5	3.88	1.92	3.21	7.4
3.48	1.80	2.86	5.2	3.91	2.05	3.26	7.5
3.48	1.86	2.90	5.0	3.92	1.92	3.07	5.4
3.49	1.75	2.90	4.7	3.92	2.00	3.22	8.0
3.49	1.77	2.92	6.1	3.93	2.00	3.11	6.8
3.50	1.80	2.87	5.9	3.93	2.00	3.20	7.4
3.50	1.81	2.83	4.0	3.93	2.00	3.22	6.6

3.94	1.93	3.31	7.2	4.20	2.18	3.42	9.5
3.94	2.03	3.19	7.3	4.28	2.14	3.38	8.2
3.96	2.00	3.24	7.1	4.28	2.37	3.40	9.2
3.96	2.12	3.40	7.7	4.29	2.19	3.65	10.8
3.98	2.00	3.31	7.5	4.32	2.24	3.40	8.3
3.98	2.10	3.36	6.6	4.35	2.20	3.67	10.2
4.01	2.01	3.23	7.0	4.36	2.15	3.58	8.9
4.06	2.08	3.27	7.2	4.38	2.20	3.68	10.8
4.10	2.02	3.32	7.4	4.38	2.23	3.47	9.0
4.13	2.03	3.37	8.1	4.39	2.14	3.55	10.1
4.18	2.08	3.36	9.2	4.45	2.28	3.46	9.4
4.20	2.09	3.44	9.5	4.49	2.36	3.61	10.2
4.20	2.09	3.47	7.8	4.66	2.35	3.73	10.1

TABLE 15. September 14, 1933.

Length in cm	Depth in cm	Height in cm.	Weight in gm	Length in cm.	Depth in cm.	Height in cm	Weight in gm
2.50	1.31	2.14	2.3	3.10	1.54	2.56	4.0
2.53	1.16	2.14	2.2	3.10	1.55	2.60	3.5
2.54	1.23	2.17	1.8	3.11	1.53	2.61	3.7
2.66	1.25	2.23	2.4	3.12	1.48	2.65	4.2
2.70	1.36	2.23	2.4	3.12	1.55	2.58	4.2
2.75	1.41	2.32	2.9	3.12	1.58	2.65	4.1
2.78	1.40	2.33	3.1	3.14	1.59	2.59	4.4
2.80	1.35	2.30	2.8	3.15	1.55	2.61	3.7
2.81	1.29	2.35	2.5	3.15	1.57	2.67	4.7
2.81	1.46	2.46	3.0	3.16	1.54	2.60	3.7
2.82	1.38	2.37	3.1	3.16	1.68	2.61	4.2
2.83	1.38	2.33	2.8	3.18	1.53	2.73	3.7
2.83	1.46	2.37	3.3	3.18	1.61	2.66	4.0
2.86	1.41	2.35	3.0	3.19	1.48	2.63	3.4
2.89	1.46	2.41	3.3	3.19	1.53	2.72	3.6
2.90	1.48	2.38	2.9	3.19	1.57	2.76	4.4
2.91	1.34	2.41	2.9	3.19	1.70	2.58	4.1
2.91	1.44	2.44	3.2	3.20	1.58	2.65	3.8
2.91	1.44	2.51	3.9	3.21	1.56	2.66	4.2
2.92	1.44	2.41	2.8	3.21	1.57	2.67	4.2
2.92	1.44	2.45	3.6	3.22	1.64	2.65	3.8
2.92	1.45	2.43	3.5	3.23	1.66	2.71	1.0
2.93	1.50	2.42	3.3	3.23	1.78	2.69	4.5
2.96	1.48	2.46	3.1	3.24	1.58	2.76	4.1
2.97	1.50	2.56	3.7	3.24	1.61	2.63	4.2
2.98	1.50	2.52	3.7	3.25	1.60	2.66	4.5
2.99	1.46	2.49	3.1	3.25	1.63	2.70	3.7
3.00	1.44	2.49	3.2	3.26	1.57	2.73	4.1
3.01	1.44	2.54	3.1	3.26	1.62	2.73	4.2
3.01	1.46	2.44	3.4	3.26	1.63	2.75	4.8
3.02	1.54	2.60	3.7	3.26	1.64	2.69	4.1
3.03	1.46	2.49	2.9	3.27	1.64	2.70	4.6
3.05	1.50	2.51	3.0	3.27	1.80	2.70	5.0
3.05	1.51	2.60	3.7	3.28	1.58	2.77	4.9
3.06	1.61	2.65	4.6	3.28	1.67	2.83	4.8
3.08	1.56	2.56	3.6	3.29	1.56	2.73	3.5
3.09	1.53	2.55	3.6	3.29	1.61	2.74	4.8
3.10	1.52	2.57	3.7	3.29	1.61	2.80	4.1
3.10	1.53	2.52	3.7	3.30	1.65	2.77	4.4
3.10	1.53	2.53	3.3	3.30	1.71	2.72	4.5

Length in cm	Depth in cm	Height in cm.	Weight in gm	Length in cm	Depth in cm	Height in cm	Weight in gm
3.30	1.74	2.77	5.2	3.66	1.92	2.99	5.1
3.32	1.68	2.80	4.9	3.68	1.72	3.03	5.2
3.32	1.70	2.62	4.0	3.70	1.94	3.11	6.9
3.31	1.69	2.80	5.0	3.71	1.88	3.12	6.8
3.35	1.81	2.87	5.6	3.71	1.98	3.08	7.5
3.36	1.62	2.76	4.8	3.73	1.90	3.02	6.9
3.36	1.62	2.80	4.3	3.73	1.99	3.10	7.0
3.37	1.73	2.91	5.1	3.74	1.97	3.11	7.3
3.37	1.76	2.74	5.0	3.75	1.96	3.10	7.7
3.39	1.64	2.78	5.0	3.76	2.02	3.04	7.4
3.39	1.68	2.77	4.8	3.77	1.95	3.21	7.5
3.40	1.74	2.75	4.8	3.78	1.86	3.10	5.7
3.42	1.72	2.78	4.6	3.79	1.99	3.06	7.1
3.43	1.69	2.88	5.1	3.82	1.85	3.09	6.0
3.43	1.71	2.81	4.2	3.82	1.98	3.05	6.9
3.44	1.61	2.81	5.0	3.83	1.92	3.11	6.3
3.44	1.62	2.83	5.0	3.83	1.96	3.08	5.2
3.45	1.68	2.80	4.3	3.83	2.01	3.11	6.6
3.45	1.74	2.84	5.7	3.88	1.83	3.24	6.2
3.46	1.78	2.75	4.5	3.88	2.02	3.12	7.1
3.47	1.70	2.91	4.7	3.91	2.03	3.20	7.0
3.48	1.88	2.83	5.2	3.92	1.90	3.20	7.0
3.52	1.70	2.93	5.2	3.99	2.02	3.16	7.7
3.52	1.75	2.88	4.8	3.99	2.03	3.18	7.0
3.52	1.85	2.90	5.7	4.00	1.96	3.31	7.9
3.53	1.73	2.91	5.0	4.01	1.98	3.31	7.5
3.57	1.83	3.03	5.6	4.02	2.05	3.35	7.2
3.58	1.70	2.88	4.8	4.03	2.03	3.31	7.2
3.59	1.78	2.91	4.8	4.03	2.12	3.30	7.9
3.62	1.75	2.99	6.1	4.10	2.04	3.35	8.4
3.62	1.86	2.98	5.1	4.13	1.97	3.30	7.0
3.63	1.86	3.03	6.6	4.18	2.13	3.57	10.7
3.64	1.80	2.97	5.3	4.22	2.10	3.36	9.0
3.65	1.87	3.02	5.5	4.22	2.22	3.44	8.9

SOME NOTES ON *MUSCULIUM HETERODON* (PILSBRY),
A FRESHWATER BIVALVE

II. THE GILL, THE BREEDING HABITS AND
THE MARSUPIAL SAC

By

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(With 17 figures in text)

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The process of the rearing of their young by all the species of Unionidae and Sphaeriidae, and by some marine eulamellibranchia is well known: e. g. in the case of *Ostrea edulis*, the eggs are brought up within the mantle cavity to the larval stage capable of free swimming (HORST 1882); in that of *Entovalva mirabilis*, by a fusion of the posterior end of the two halves of the mantle, a bell-shaped brood cavity is formed, in which the embryos remain until they reach the trochophore stage (VOELTZKOW 1891); in that of *Unio* and *Anodonta*, the glochidia are delivered after almost the whole completion of their embryonic stage in the interlamellar space of the outer gill (GOETTE 1891, HARMS 1908, and LEFEVRE and CURTIS 1910); and in that of the Sphaeriidae, the embryos are nourished in the marsupial sac, which is a closed sac formed in the supra-branchial chamber of the inner gill (inner branchial chamber), until they have grown through in the earliest stage of complete mussels with two valves, the whole stage of free swimming being entirely non-existent.

Such a peculiar breeding habit in the Sphaeriidae has been studied by many investigators. Of these, POYARKOFF (1910), SCHERESCHEWSKY (1911), and WASSERLOS (1911) in *Sphaerium corneum*, and GROENEWEGEN (1926) in *Sphaerium rivicola* studied the histological structure, origin, and function of the marsupial sac; and THIEL (1924, '28, '30) in *Sphaerium corneum* and FOSTER (1932) in *Sphaerium solidulum* carried out investigations in relation to the growth, reproduction, and life history, chiefly from the view-point of the seasonal change.

My object has been also to determine the details with regard to the breeding habits and the marsupial sac of the Japanese species, *Musculium heterodon*. The method used is the same as that stated in my previous paper (1934).

In this connexion, I wish to express my appreciation of the guidance received from Prof. Dr. E. NOMURA throughout the present work. My thanks are also due to Assist.-Prof. I. MOTOMURA for his advice.

THE STRUCTURE OF THE GILL.

The gills of *Musculium heterodon*, with the exception of their position and size, which are the peculiar characteristics of the Sphaeriidae, conform to the so-called "Anodonta-type" (Fig. 1).

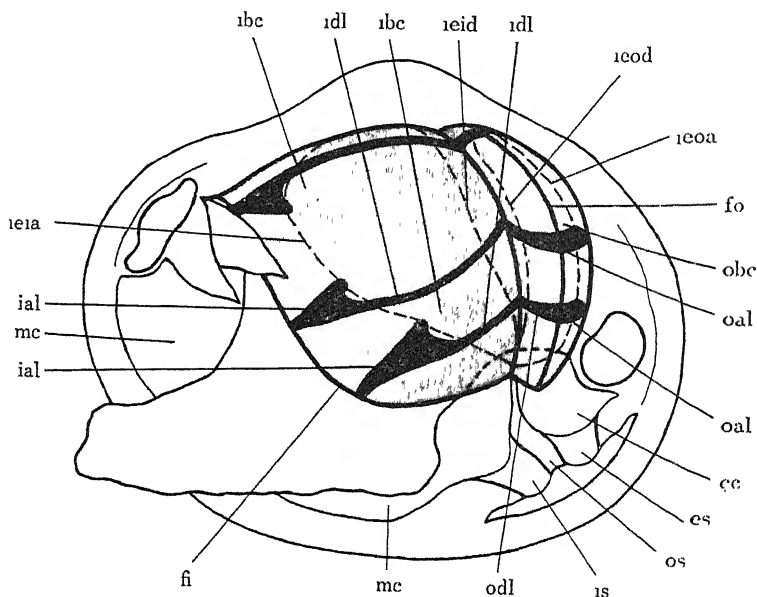


Fig. 1 Schematic representation of topographical relation between organs in mantle cavity of *Musculium heterodon*, especially to illustrate gross structure of gill. Left side view. *cc* cloacal chamber, *es* exhalant siphon, *fi* free edge of inner gill, *fo* free edge of outer gill, *ial* ascending lamella of inner gill, *ibc* inner branchial chamber, *idl* descending lamella of inner gill, *ieia* insertion edge of ascending lamella of inner gill, *ieid* insertion edge of descending lamella of inner gill, *ieoa* insertion edge of ascending lamella of outer gill, *ieod* insertion edge of descending lamella of outer gill, *is* inhalant siphon, *mc* mantle cavity, *oal* ascending lamella of outer gill, *obc* outer branchial chamber, *odl* descending lamella of outer gill, *os* oblique septum of siphon.

Each branchial lamella consists of branchial filaments, which run parallel to each other forming a plate connected by interfilamental junctions, and the corresponding filaments of the descending and ascending lamellae are connected by interlamellar junctions (Fig. 2). The gill is therefore com-

posed of two filamental spaces forming a lattice-like system. one the intrafilamental space which contains blood, and the other the interfilamental space which is the water passage.

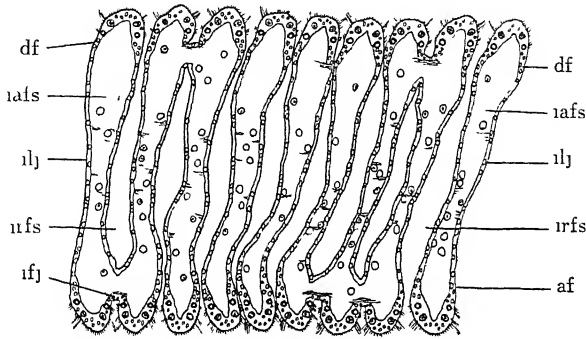


Fig. 2 Section perpendicular to lamellae of inner gill, to illustrate relation between filaments and junctions $\times 66$. Blood corpuscles seen in intrafilamental space af ascending filament, df descending filament, iafs intrafilamental space, ifj interfilamental junction, ilj interlamellar junction, irfs interfilamental space

The wall of each filament is a unicellular layer. The layer facing the mantle cavity is composed of columnar and sphenoidal cells of different kinds (Fig. 3). The basal cells forming the sides of the filament decrease gradually in height and become flattened, and thus continue to form not only the remaining wall of the filament, but also the walls of the interlamellar and interfilamental junctions (Fig. 2).

Each inner gill is large, triangular in shape, and nearly covers the whole visceral sac on respective sides of it. Its insertion edges run from the dorsal portion of the visceral sac, postero-ventrally, towards the siphon, and are continuous to the oblique septum, which completely separates the branchial chamber from the mantle cavity. The proportion of the descending to the

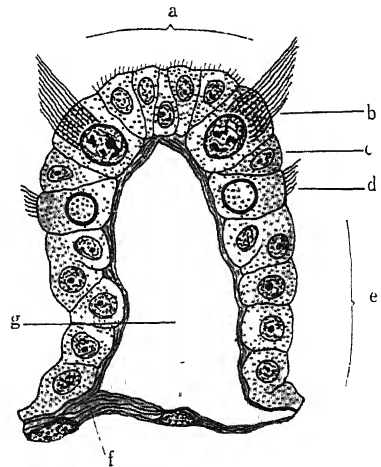


Fig. 3. Cross section of filament, to illustrate cellular arrangement. $\times 800$. a frontal cells, b latero-frontal cell, c inter-connective cell, d lateral cell, e basal cells, f basement membrane, g intrafilamental space

ascending lamella is about 4:1 in length of filaments as the anterior region, 3:1 at the middle, and 1:1 at the posterior.

Each outer gill is much smaller than the inner, and only covers rather less than the posterior third of the visceral sac. The proportion of the descending lamella to the ascending is about 1:4 all through.

The supra-branchial or interlamellar chamber of the present species is very spacious, being lined with the descending and ascending lamellae, by the wall of the visceral sac, which is widely spread between the insertion edges of both lamellae, and by the interlamellar bridge, which is the uppermost series of interlamellar junctions, being depressed so strongly downwards that it nearly approaches the free edge of the gill. This chamber is, therefore, called the branchial chamber in this paper, and,

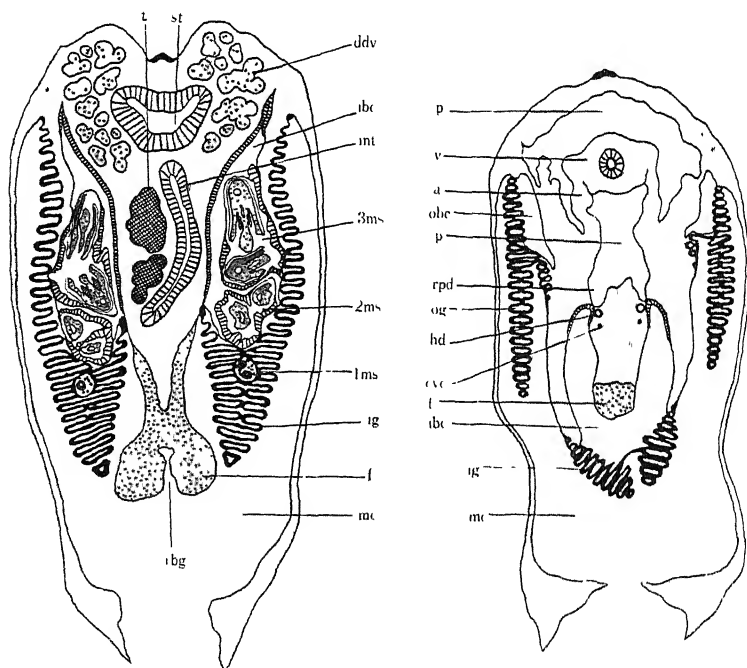


Fig. 4. Vertical cross sections $\times 15$. Left, through middle region of body, to illustrate three marsupial sacs in different developmental stages. Right, through heart and most posterior end of foot, to illustrate common inner branchial chamber and outer branchial chamber proceeding to unite with it, just in front of cloacal chamber. *1ms* primary marsupial sac, *2ms* secondary marsupial sac, *3ms* tertiary marsupial sac, *a* auricle, *cvc* cerebro-visceral connective, *ddv* digestive diverticula, *f* foot, *hd* hermaphroditic duct, *ibc* inner branchial chamber, *ig* inner gill, *int* intestine, *mc* mantle cavity, *obc* outer branchial chamber, *og* outer gill, *p* pericardium, *rbg* recess of vestigial byssus gland, *rpd* reno-pericardial duct, *st* stomach, *t* testis, *v* ventricle.

correspondingly, that of the inner gill the inner branchial chamber, and that of the outer gill the outer branchial chamber.

The embryos are bred in the inner branchial chamber which is much more spacious than the outer one. These two pairs of branchial chambers unite, posteriorly, with each other, to form the cloacal chamber, which opens to the outside of the body through the exhalant siphon (Fig 1 and 4).

Many spherical blood corpuscles are usually found in the intrafilamental space, especially, of the interlamellar junctions. These corpuscles are also found in the blood vessels of the body or in the haemocoel together with the connective tissue cells. Two kinds of blood corpuscles may be distinguished according to the different structures of their nuclei (Fig. 5), but I am unable to discuss the details of this point at present.

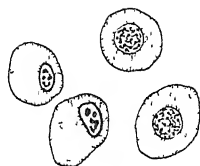


Fig 5 Blood corpuscles
× 800

THE BREEDING HABIT

The breeding season of the species under discussion appears to continue throughout the year, because, in all the specimens in my monthly collections, the marsupial sacs have always been found to have embryos enclosed within them.

As to the development of the marsupial sac three different stages may be distinguished, viz. the primary, secondary, and tertiary, in this order of formation. The primary marsupial sac is the smallest and is of the latest formation, containing the developing eggs in cleavage. At the beginning of this formation, three or four smaller primary sacs are usually found, but they unite later and form a larger primary sac. The secondary marsupial sac contains the embryos at a somewhat earlier stage, which nearly corresponds to the gastrula or larval stage of other families. The tertiary marsupial sac is the largest of the three, and is of the earliest formation, containing the embryos of later stages which have grown almost into the young mussels.

Besides the marsupial embryos, which are enclosed in the marsupial sacs, there are found some extra-marsupial embryos, which are perfect mussels and have been readily liberated from the tertiary marsupial sac, and remain making jerky movements in the inner branchial chamber for a time before their delivery outside the body. The extra-marsupial embryo

measures 1.5–2.5 mm. in length, and I am not able to distinguish it from the young mussel of the same size already born. No mussel of such a length ever attains sexual maturity.

The secondary and tertiary marsupial sacs are present throughout the year; but the appearance of the extra-marsupial embryos is to the spring and the autumn, and these are to be born sooner or later during succeeding days. The primary marsupial sac is newly formed, in most cases, while the birth of the extra-marsupial embryos is proceeding, though rarely, after the completion of this process. As already stated in my previous paper, even though the period of the sexual maturation in the testis extends throughout the year, yet the seasonal differences are observable. The spermatogenesis is most active in early spring and in early autumn. In June and October, mature spermatozoa are most plentiful, but in winter only a small number of them are seen in the shrunken testis. In the ovary, the finding of the primary oöcytes is limited to spring and autumn, although the growing oögonia are always seen throughout the year.

In mussels measuring 3–7 mm. in length, the median lobe of the testis is well developed, but in those measuring more than 8 mm., it is much

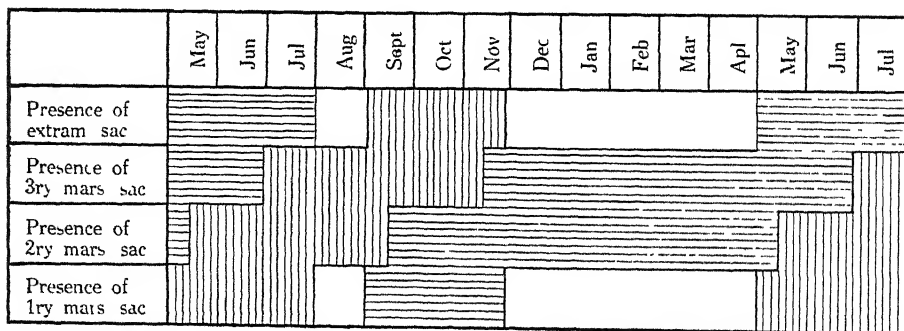


Fig. 6 Diagram to show presence of embryos of different stages according to different months. Marsupial embryos enclosed in primary, secondary and tertiary marsupial sacs and extra-marsupial embryos present in May, June, July, September, October, and November. In the remaining months, marsupial embryos enclosed only in secondary and tertiary marsupial sacs present. Development of spring breed represented by horizontal parallel lines, and that of autumn breed by vertical parallel lines.

shrunken and only the lateral lobes maintain their existence. The oögenesis is, on the contrary, most vigorous in mussels above 8 mm. in length. The protandrous maturation of this species may thus be distinctly suggested.

From the maturity of the gonads above mentioned, it is obvious that

the period of reproduction occurs twice a year. The eggs fertilized in spring are enclosed in a primary marsupial sac. This is enlarged with the development of the embryos, and becomes a secondary marsupial sac. This also continues to enlarge and becomes a tertiary marsupial sac. Each marsupial embryo enclosed in this sac grows and becomes liberated into the inner branchial chamber as an extra-marsupial embryo in the autumn of the same year. The same sequence of events occurs also in the case of the eggs fertilized in autumn, save in the slowness in growth in the period of hibernation in the winter and in the birth in the spring of the next year. Fig. 6 is prepared in order to illustrate the connexion between the month and the presence of embryos at different stages. The formation of the primary marsupium usually occurs at three or four different times in one period of reproduction.

GROENEWEGEN (1926) reports that the fertilized eggs of *Sphaerium rivicola* are found from the middle of May until the end of autumn in Leipzig. THIEL (1930) reports two periods of reproduction, in early summer, and autumn, in reference to *Sphaerium corneum* in Hamburg. According to FOSTER (1932), *Sphaerium solidulum* reproduces in two seasons of a year, but in Illinois the period of maximum reproduction occurs in the winter months. These investigators coincide invariably in recognizing the double occurrence of the reproductive period throughout the year, although the season of maximum reproduction may differ in different species or in different localities. In my case, the fertilization of the eggs and the birth of the young mussels of *Musculium heterodon* occurred in the two seasons, spring and autumn, in Sendai; the fertilization is most remarkable in June and the birth in October.

The young mussels which have been brought forth in the spring (spring breed) continue to grow during the summer, attain their first sexual maturity in the autumn, and their young mussels are born in the spring, next year. Soon after this first delivery, some new eggs are enclosed in a new marsupial sac, and continue to grow until the autumn, in which they are born. Those which have been brought forth in the autumn (autumn breed) grow successively, except during the period of hibernation, attain their first sexual maturity in the spring, next year, and reproduce their young in the autumn. In the second year, thus, both breeds are capable of reproduction in the two periods, spring and autumn (Fig. 7). The natural death of large, aged mussels occurs twice a year, in April and November. The exact duration of natural life of this species, however, is quite unknown to me at present, because of want of success in culturing

mussels in laboratory water.

The mussels which have attained their first sexual maturity measure more than 2.5 mm. in length, and those which are breeding their embryos are more than 3 mm. The size and number of embryos, forming a brood, differ in different individual mussels, even when the marsupial sacs are seemingly of simultaneous formation and of the same size. Differences in size are also perceptible to some extent in the embryos, even in a given marsupial sac of a given individual. The number of embryos on either side of the body is usually equal in a given individual, but occa-

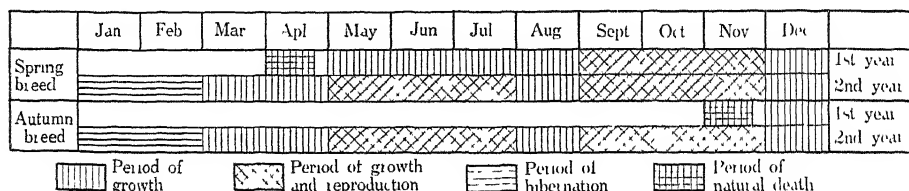


Fig 7. Diagram to illustrate the relation between periods of growth, hibernation, reproduction, and natural death

sionally a difference of two or three embryos happens to be found. The number of embryos in a brood is always smaller in the mussels measuring 3-5 mm. in length than in the larger mussels. Table 1 is given in order to show that the number of embryos, which were contained in the tertiary marsupial sac, increased with the growth of the mother.

TABLE 1.

Changes in number of embryos in tertiary sac, invariably on left side of mussels measuring 6 mm or more in length, collected on October 15, 1933.

Length of adult mussels in mm	6±0.2	7±0.2	8±0.2	9±0.2
Maximum number of embryos	8	10	10	16
Minimum number of embryos	3	5	5	8
Average number of embryos from 20 adult mussels	4.7	6.4	7.6	10.5

THE STRUCTURE AND DEVELOPMENT OF THE MARSUPIAL SAC

The marsupial sac is the breeding organ in the Sphaeriidae, which was found at first by JACOBSON (1828) in *Sphaerium corneum*. It begins to form in the anterior floor of the inner branchial chamber, being stim-

ulated probably by the arrival of the fertilized eggs. As already stated, it passes in its development through the three stages of the primary, secondary and tertiary sacs (Fig. 4)

At the very beginning of the formation of the primary sac, the eggs come first into the interspace between the interlamellar junctions, and then the walls of the adjacent junctions are eroded or destroyed by some mechanism, which is unknown to me, but it appears to be a fact that the erosion or destruction is aided by the action of the blood corpuscles, because the wall, which begins to enclose the eggs, invariably thickens, especially as the result of an addition of a number of blood corpuscles, and the original primary sacs are formed by the cells of this thickened wall (Fig. 8).

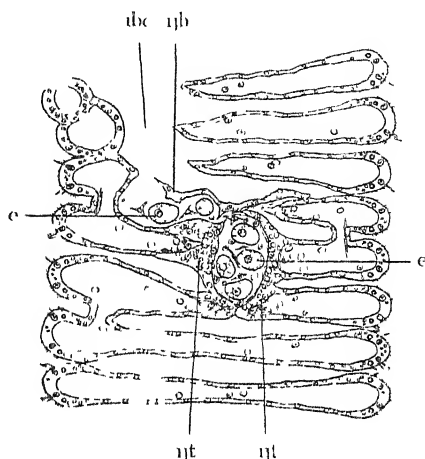


Fig. 8. Section perpendicular to branchial filaments, to show formation of primary marsupial sac $\times 66$ *e* fertilized egg, *ibc* inner branchial chamber, *yb* wall of interlamellar junction broken, *yt* wall of interlamellar junction thickened

At first, usually three or four small primary sacs are formed independently of each other, each sac containing four or more eggs. Later, these sacs fuse with each other in the progress of growth, and form a large primary marsupial sac, which contains often more than fifteen embryos. By this time, it swells out distinctly into the inner branchial chamber. Its wall is a thin, unicellular epithelium with its basement membrane, and is continuous with that of the interlamellar junctions, showing a structure similar to the latter (Fig. 9). There are always found, besides the developing eggs, a number of blood corpuscles that have wandered into the primary sac from the intrafilamentary space. The complete primary marsupial sac thus formed, still continues to enlarge, and at last comes to be called the secondary marsupial sac.

The structure of the secondary marsupium is remarkably different from that of the primary. Its wall consists of two different layers, outer and inner, apart from each other, lining the interspace, except at the fusing points of the two layers which are frequently seen (Figs. 10 and 11 *f*). The outer layer is of a similar structure to the wall of the primary sac.

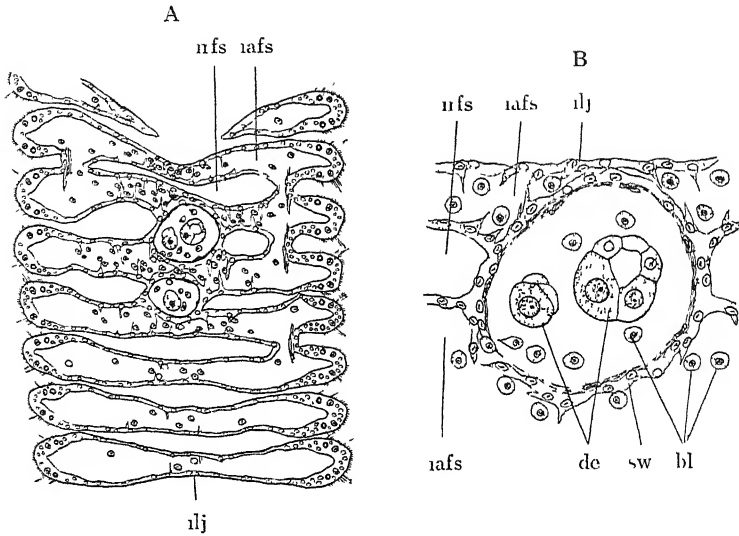


Fig 9 Section perpendicular to branchial filaments, showing position (A) and structure (B) of primary marsupial sac. A $\times 66$, B $\times 270$. *bl* blood corpuscles, *de* developing eggs, *iafs* intrafilamental space, *ilj* interlamellar junction, *nfs* interfilamental space, *sw* wall of primary marsupial sac.

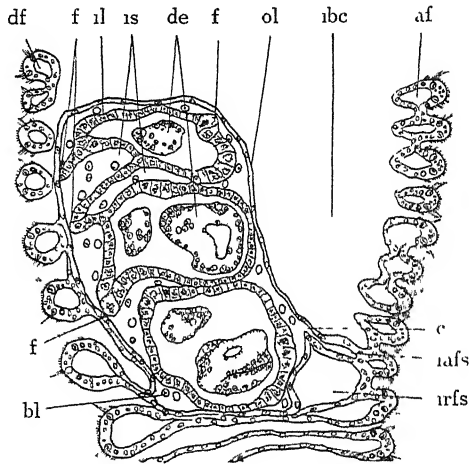


Fig 10 Section perpendicular to branchial filaments, showing secondary marsupial sac. $\times 66$. *af* ascending filament, *bl* blood corpuscle, *c* connexion of interspace between inner and outer layers of secondary marsupial sac with intrafilamental space of interlamellar junction, *de* developing embryos, *df* descending filament, *f* fusing point of inner and outer layers of secondary marsupial sac, *iafs* intrafilamental space, *ibc* inner branchial chamber, *il* inner layer of secondary marsupial sac, *irfs* interfilamental space, *is* interspace between inner and outer layers of secondary marsupial sac, *ol* outer layer of secondary marsupial sac.

The inner layer is irregularly folded, and composed of various cells (Fig. 11). Of these cell components, at least, three types are distinguishable: viz. the ordinary type, the compound type, and the transition type from ordinary to compound.

The cells of ordinary type (Fig. 11 *of*) are arranged unicellularly and form the thinner parts near the fusing points of the inner and outer layers (Fig. 11 *f*). Each cell is furnished with a distinct cell membrane, with an amount of dense cytoplasm and with an oval or spherical nucleus, which contains a nucleolus and granular chromatin.

The cells of compound type (Fig. 11 *og*) are also arranged unicellularly and form the thicker parts of the inner layer, sometimes measuring 90 μ .

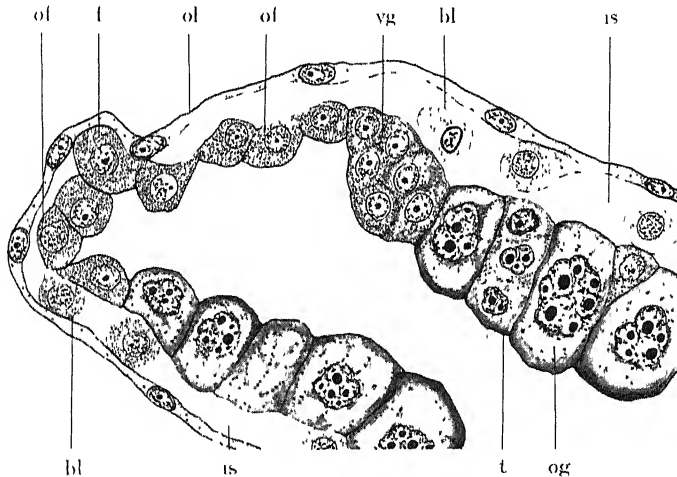


Fig. 11 Section of wall of secondary marsupial sac, to show its cellular structure $\times 600$ *bl* blood corpuscle, *f* cell in fusing point of inner and outer layers, *is* interspace between inner and outer layers, *of* cell of ordinary type forming inner layer, *og* cell of compound type forming inner layer, *ol* outer layer, *t* compound cell of transition type, *vg* cells of ordinary type aggregating into mass, showing transition to cell of compound type

Each member of this type is almost columnar or cubical, being furnished with a distinct outer membrane, with an amount of cytoplasm highly vacuolated, and with a compound nucleus. This compound nucleus consists naturally of a number of ordinary nuclei fused with each other into an irregularly shaped mass. In most cases, the membrane of each ordinary nucleus is fading or has faded away, the nucleolus, which is much increased in size, remaining.

As to the cells of the transition type, two cases are shown in Fig. 11.

One is the case (*yg*), in which cells of the ordinary type are aggregated into a syncytial mass with only an outer membrane. The other is the case (*t*), in which the behaviour of the cell resembles that of the compound cell, but the cell contains three compound nuclei and somewhat dense cytoplasm without vacuoles. If the nuclei were fused into one and the cytoplasm were vacuolated, it would be merely a compound cell.

The interspace (Figs. 10 and 11 *is*), which is found between the inner and outer layers, is continuous with the intrafilamental space of the interlamellar junctions, to which the marsupial sac is attached. The wandering blood corpuscles may, therefore, be introduced directly into the interspace of the marsupial sac from the intrafilamental space of the junctions.

The secondary marsupial sac assumes rather a pear-shaped form, bulging out into the inner branchial chamber with its wider end and being attached to the interlamellar junctions at its narrower end, which may be called the sac stalk (Fig. 12 *st*).

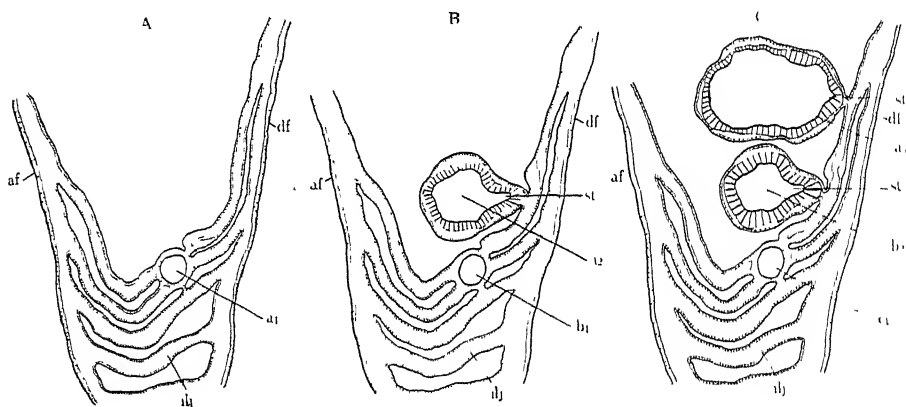


Fig. 12 Schema illustrating growth of marsupial sac. Developmental stages pass A to C. *a*₁₋₃ primary marsupial sac of A becomes secondary marsupial sac of B and then tertiary marsupial sac of C. *b*₁₋₂ newly formed primary marsupial sac of B becomes secondary marsupial sac of C. *c*₁ newly formed primary marsupial sac. *af* ascending filament, *df* descending filament, *ilj* interlamellar junction, *st* sac stalk.

Originally, the primitive secondary sac has its own sac stalk, attached to the interlamellar junctions where it originated, being then termed the primary marsupium. This sac stalk is no longer connected with the original attaching point, when a second sac stalk is established at a point where the sac is newly attached to the descending lamella of the inner gill.

Then the third sac stalk forms, and the second disappears. Thus, the secondary sac gradually moves upwards along the descending lamella, because this sac is enlarged and so requires more space in the upper part of the inner branchial chamber (Fig. 12). As a rule, the sac stalk is never formed in conjunction with the ascending lamella.

The sac stalk is reformed in the following manner: the upper wall of the interlamellar junctions, next to the original attaching point at first becomes thickened by the addition of a number of blood corpuscles. This thickened wall touches the wall of the secondary marsupial sac, which is pressing upwards, and then both walls fuse with each other. Thus the reformation of a new sac stalk is accomplished by the addition of blood corpuscles to the inner layer of the sac.

The secondary sac thus occupying the uppermost position in the inner branchial chamber continues to be further enlarged in harmony with the growth of the embryos, till it is termed the tertiary marsupial sac.

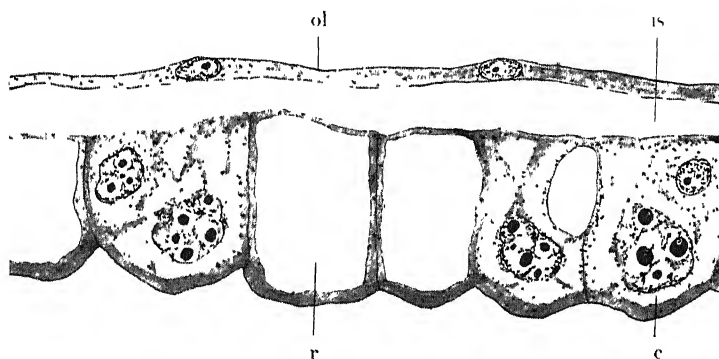


Fig. 13. Section of wall of tertiary marsupial sac, to show its cellular structure. $\times 600$ *c* compound cell, *is* interspace between inner and outer layers, *ol* outer layer, *r* residue of compound cell

The tertiary marsupial sac contains the embryos approximating to the shape of the adult. Its wall is irregularly folded, owing probably to a rapid growth of the embryos. The structure of the wall is almost similar to that of the secondary sac (Fig. 13). Two peculiarities may, however, be described in connexion with the inner layer of the tertiary sac. One is the presence of the vacant cells (*r*), which are the residue of the compound cells, with their contents gone, only their peripheral portions remaining. The other is the transparency to a larger extent of the inner layer, where it is composed of compound and vacant cells. This transparency is due

to a thinning of the layer much expanded, and we can see clearly the embryos within the sac through its wall

THE ORIGIN OF THE INNER LAYER OF THE MARSUPIAL SAC AND OF
THE NUTRIMENT LAYER OF THE INNER BRANCHIAL CHAMBER,
AND THE NOURISHMENT OF THE EMBRYOS

Though it has been confirmed for some time that the outer layer of the marsupium originates from the cells constituting the wall of the interlamellar junctions of the inner gill, yet the origin of the inner layer has been variously explained. According to POYARKOFF (1910), the inner layer originates from nothing else but from the blood corpuscles. SCHIERESCHIEWSKY (1911) is of opinion that some mesodermal cells, which are

retained in the wall of the interlamellar junctions, form this layer. GROENEWEGEN (1926) has opposed POYARKOFF's view even though he also recognizes that the blood corpuscles are the nutriment of the embryo, and is of opinion that the inner layer originates from the cells constituting the interlamellar junctions as does the outer layer. His objection is based on the point that it is impossible for the wandering blood corpuscles to adhere to the permanent epithelium, and that the transitory forms from the blood corpuscles to the cell components of the inner layer appear very unsatisfactory. My own observation concerning the origin of this inner layer supports POYARKOFF's view, because of the presence and

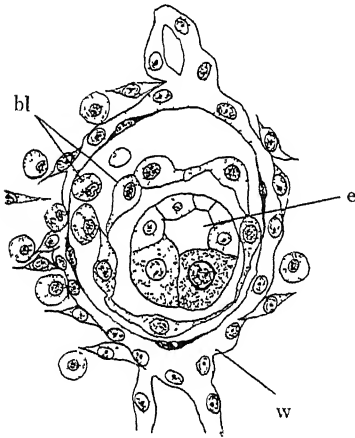


Fig. 14 Section of primary marsupial sac, to show rearrangement of blood corpuscles into inner layer of secondary marsupial sac $\times 400$. *bl* blood corpuscles, *e* developing egg, *w* wall of primary marsupial sac

rearrangement of the blood corpuscles within the primary marsupial sac round the developing embryos (Fig. 14), and of the presence of cells showing a transition from the blood corpuscles to the compound cells (Fig. 11).

The primary sac, after the complete closing of the wall, grows gradually towards the inner branchial chamber, as above mentioned, and, at the same time, the number of blood corpuscles increases within it. This increase may be caused by the wandering out of the blood corpuscles into the sac

from the intrafilamental space (Fig. 9 *bl*). These blood corpuscles begin to gather and rearrange themselves around the embryos (Fig. 14) and form there a primitive inner layer which is composed of cells of the ordinary type. It may, therefore, be positively stated that the inner layer of the secondary and tertiary sacs originates from the blood corpuscles enclosed in the primary sac. In the secondary sac, the blood corpuscles, which are introduced into the interspace between the outer and inner layers, may be used, as supplementary elements, to extend the wall in harmony with the development of the embryos. The cells at the fusing points of the two layers (Figs. 10 and 11) resemble the cells of the ordinary type of the inner layer, and probably indicate the existence of a transitory stage from the wandering blood corpuscles to the cells forming the inner layer.

That there is a secretory action on the part of the compound cells forming the inner layer of the secondary sac has already been suggested by SCHERESCHIEWSKY (1911). The secretions may be used as the nutriment of the early embryos.

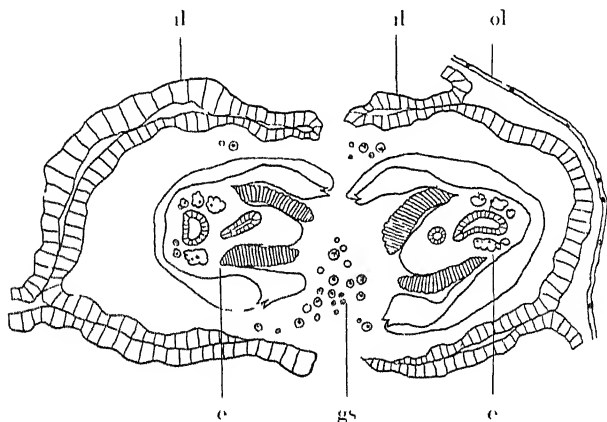


Fig. 15. Section of tertiary marsupial sac, to show globular substance within it $\times 66$. *e* marsupial embryos in later stage of development, *gs* globular substance, *il* inner layer of tertiary marsupial sac, *ol* outer layer of tertiary marsupial sac

In the tertiary marsupial sac, near the embryos of the later stage, are often found many scattered globules (Fig. 15), which were first detected and described by STEPANOFF (1865). Since then these globules have been recognized as being the food of the embryos. Two kinds of globules are found (Fig. 16); one the blood corpuscles proper, and the other the cell

contents fallen away from the compound cells. On the other hand, the presence of the residue of the compound cells reveals the gradual consumption of the matter of the inner layer. Thus, the embryos grow at the expense of the inner layer, and finally come to be called the extra-marsupial embryos.

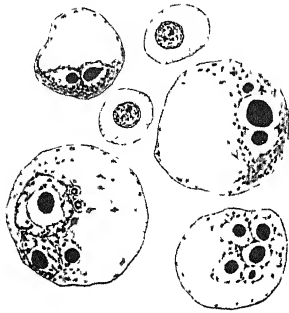


Fig. 16. Different globules highly magnified $\times 600$

In the mussels, which have passed the first birth of their young mussels, is always found, adhering to the outer surface of the wall of the visceral sac, a layer, which consists of various cells (Fig. 17), similar to those observed in the case of the inner layer of the marsupial sac. The extension of this layer

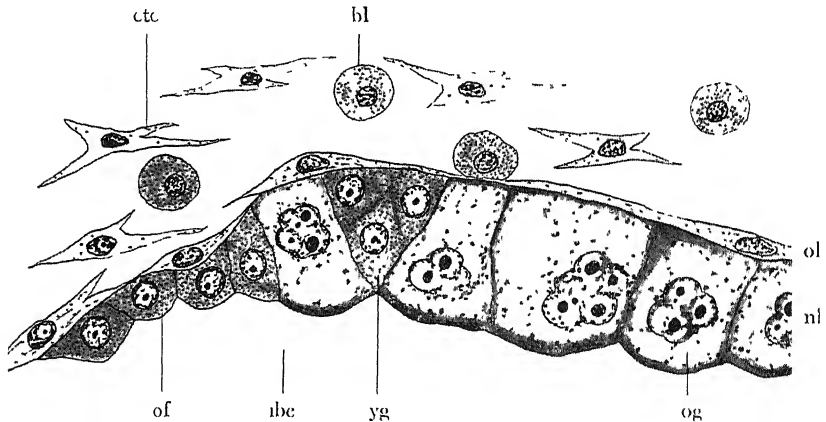


Fig. 17 Section of wall of visceral sac lining the inner branchial chamber $\times 600$
bl blood corpuscle, *ctc* connective tissue cell, *ibc* inner branchial chamber, *nl* nutrient layer, *of* cells of ordinary type forming nutrient layer, *og* cell of compound type forming nutrient layer, *ol* ordinary epithelium of visceral sac, *yg* cells of ordinary type aggregating into mass showing transition to cell of compound type

is limited to the area, where it lines the inner branchial chamber, and from its availability, as a source of nourishment, it may be called the nutrient layer. The nutrient layer is never found in mussels younger than those which are preparing for the first birth.

The formation of the nutrient layer is nearly similar to that of the inner layer of the marsupium. At first, the blood corpuscles migrate from the haemocoel of the visceral sac, and form a unicellular layer consisting

of cells of the ordinary type, beginning from the postero-dorsal side of the area, and finally this layer extends all over the limited surface. While the layer of ordinary cells is extending outwards, in the area of its older formation, each member is being transformed into the compound cell, and by the time of the arrival of the extra-marsupial embryos in the inner branchial chamber, the whole area of the full-extended nutriment layer becomes composed completely of compound cells and of their residues. After the decay of the residual compound cells, their original position is supplemented by cells of the ordinary type, which are clearly blood corpuscles which have migrated from the haemocoel of the visceral sac. By the further addition of the blood corpuscles, they form there syncytial cell masses, which are finally transformed into compound cells. If the inner layer clearly originated from the epithelial cells of the interlamellar junctions, the nutriment layer might also originated from the outer epithelial cells of the visceral sac. But this is never the case, and the result of my investigation decisively points to the conclusion that both the inner layer of the wall of the marsupial sac and the nutriment layer of the inner branchial chamber originate invariably from the blood corpuscles.

The nutriment layer, thus formed, may supply nourishment to the extra-marsupial embryos, as the inner layer of the marsupial sacs does to the marsupial embryos. It may therefore be concluded here that all through its developmental stages, *Musculium heterodon* is nourished at the expense of the blood corpuscles of the mother.

SUMMARY AND CONCLUSIONS

1) A study of the process of supplying nutrition to the embryos of *Musculium heterodon* has been carried out.

2) The structure of the gill is of the "Anodonta-type", but the inner gill is much larger than the outer, and contains a remarkably spacious, inner branchial chamber.

3) The marsupial sacs, which enclose the embryos and afford nutriment to them, are formed in the inner branchial chamber.

4) The embryos, while they are receiving nutriment in the marsupial sac, are called the marsupial embryos.

5) The embryos, which are outside the marsupial sac, but which are retained in the inner branchial chamber, are called the extra-marsupial embryos.

6) In Sendai, the fertilization of the eggs and the birth of the young mussels appear in the two seasons, spring and autumn.

7) The spring breeds bring forth the young mussels in the spring of the next year, and the autumn breeds in the autumn of the next year.

8) Three stages of development of the marsupial sac are distinguished in the order of formation.

9) The primary marsupial sac is of the latest formation, and is the smallest, originating from the interlamellar junctions in the anterior floor of the inner branchial chamber. It contains developing eggs in the stages of cleavage. Its wall is single-layered.

10) The primary marsupium begins to form as soon as the fertilized eggs have reached the interspace between the interlamellar junctions.

11) At first three or four smaller primary marsupial sacs are formed at different times in one period and independently of each other.

12) While these smaller sacs are enlarging, they fuse with each other and form a larger primary marsupial sac.

13) The secondary marsupial sac is attached to the descending lamella of the inner gill, being dislocated upwards from its original attachment to the floor of the inner branchial chamber, and contains embryos in the stages later than gastrula. Its wall is double-layered, outer and inner.

14) The tertiary marsupial sac is of the earliest formation, and is the largest, being dislocated to the uppermost part of the inner branchial chamber, and attached also to the descending lamella. The embryos contained within it nearly attain the shape of the adult. Its wall is also double-layered, outer and inner.

15) The outer layer of the secondary and tertiary marsupial wall is actually the wall of the primary marsupium, which was originally constituted of the epithelial cells of the interlamellar junctions, and which bulged out into the branchial chamber.

16) The inner layer of the secondary and tertiary marsupial wall is composed of cells of various types, viz. cells of the ordinary type, those of the compound type, and those of a transition type between the two.

17) Vacant cells or residues of compound cells are found in the inner layer of the tertiary marsupium.

18) The inner layer originates from the blood corpuscles of the mother, which have wandered into the primary marsupium.

19) The inner layer, later, is supplemented by the blood corpuscles which were introduced into the sac, while the secondary marsupium was rising upwards along the descending lamella of the inner gill.

20) The rising of the secondary marsupium is effected by the re-formation of a new sac stalk and the disappearance of an old sac stalk,

the new attaching point to the interlamellar junctions being altered successively from the lower part to the upper part of the branchial chamber.

21) The nutriment layer of the inner branchial chamber originates from the blood corpuscles migrating from the haemocoel of the visceral sac to its outer surface.

22) The marsupial embryos are supplied with their nutriment from the inner layer of the marsupial sacs, and the extra-marsupial embryos from the nutriment layer of the inner branchial chamber.

WORKS AND PUBLICATIONS REFERRED TO

- FOSTER, T. D. 1932. Observations on the Life History of a Fingernail Shell of the Genus *Sphaerium*. Jour. Morph., 53.
- GOETTE, A. 1891. Bemerkungen über die Embryonalentwicklung der *Anodonta piscinalis*. Zeits. wiss. Zool., 52.
- GROENEWEGEN, J. A. W. 1926. Über den Bau und die Entwicklung der Bruttaschen von *Sphaerium rivicola* L.M. Zeits. Morph. Okol. Tiere, 5.
- HARMIS, W. 1908. Die postembryonale Entwicklung von *Unio pictorum* und *Unio tumidus*. Zool. Anz., 32.
- HORST, R. 1882. On the Development of the European Oyster. Quar. Jour. Micro Sci., 22.
- JACOBSON, L. L. 1828. Cycladens Anatomiske Undersølgelse, Kgl. Dansk. Selsk. Naturvid. Afhandl., 3.
- LEFEVRE, G. and CURTIS, W. C. 1910. Reproduction and Parasitism in the Unionidae. Jour. Exp. Zool., 9.
- OKADA, K. 1931. Some Notes on *Musculium heterodon* (PILSBRY), a Freshwater Bivalve. I. The Genital System and the Gametogenesis. Sci. Repts. Tôhoku Imp. Univ., Biol., 9.
- POYARKOFF, E. 1910. Incubation des embryons et régénération des branchies chez *Cyclas*. Archiv. Zool. Exp., 5.
- SCHERESCHESKY, H. 1911. Struktur und Bildung der Bruttaschen bei *Cyclas cornea*. Zeits. wiss. Zool., 98.
- STEPANOFF, P. 1865. Über die Geschlechtsorgane und die Entwicklung von *Cyclas cornea*. Archiv. Natur., 31.
- THIEL, M. E. 1924. Versuch, die Verbreitung der Arten der Gattung *Sphaerium* in der Elbe bei Hamburg aus ihrer Lebensweise zu erklären. Arch. f. Hydrobiol., 4.
- THIEL, M. E. 1928. Zur Biologie unserer Süßwasser-Muscheln. Zeits. Morph. Okol. Tiere, 13.
- THIEL, M. E. 1930. Untersuchungen über den Einfluss der Abwässer von Hamburg-Altona auf die Verbreitung der Arten der Gattung *Sphaerium* in der Elbe bei Hamburg. Inter. Rev. gesam. Hydrobiol. Hydrogr., 1930.
- VOELTZKOW, A. 1891. *Entovalva mirabilis*, eine schmarotzende Muschel aus dem Darm einer Holothurie. Zool. Jahrb., Abt. Systematik, 5.
- WASSERLOS, E. 1911. Die Entwicklung der Kiemen bei *Cyclas cornea* und anderen Acephalen des süßsen Wassers. Zool. Jahrb., Abt. Anat. Ont., 31.

GALVANOTROPISM OF THE CATFISH: *PARASILURUS* *ASOTUS* (LINNÉ)¹⁾

By

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(With 3 text-figures)

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INTRODUCTION

It was already found that the catfish, *Parasilurus asotus* (LINNÉ) in an aquarium showed various but characteristic reactions to a knocking sound in advance of the occurrence of an earthquake (HATAI and ABE, 1932). Further research shows that such reactions seem to occur as the result of electrical stimulus produced by the earth currents ('32). UZUKA (1934) has studied on the responses of the fish to weak electric currents or to metallic rods, and have found similar as that first found by PARKER (1917) that the negative responses occur oftener if the stimulus is stronger, conversely the positive responses occur more frequently with weaker stimulus.

It was our next question to study the galvanotropic nature of the catfish and the results of the research are reported in the present paper.

This experiment was carried at the Asamushi Marine Biological Station from the end of February to May in 1934. During the course of this investigation I was kindly guided by Professor SHINKISHI HATAI, whom I wish to express my sincere thanks. Here I also wish to thank Assistant Professor SELJI KOKUBO and other members of the staff for their helpful suggestions and to the SAITO Gratitude Foundation for the grant with which the present investigation was made.

METHOD OF THE EXPERIMENT

A wooden aquarium of 1 metre in length, 60 cm. in breadth and 15 cm. in height was used. An iron wire-net of 60 cm. in breadth and 15 cm. in height was used for the electrodes. The bottom of the aquarium was lined 10 cm. apart for the sake of determining the exact pass ways of

¹⁾Contribution from the Marine Biological Station, Asamushi, Aomori-Ken. No. 118.

the fish. The room was darkened and a 100 w. electric lamp was hung about 1 metre high from the water surface in the middle of the aquarium.

During the experiment, the aquarium was filled with fresh water 4 cm. deep. The catfish was placed in the middle part of the aquarium facing its head to a given direction and then a direct current was transmitted, first weak but gradually increased till the fish showed locomotion. The minimum intensity of current by which the fish shows locomotion is taken as the value for the galvanotropismic response. The minimum current which caused the fish to motion was kept unchanged and the directions of locomotion of the fish was noted. The resistance of water between both the electrodes was about 5000 to 7000 ohms at 4.3°C to 8.0°C.

RESULTS OF THE EXPERIMENTS

1. General description on the influence of an electric current to the catfish.

When the catfish is tested by the method above stated, the fish does not show any visible reaction to the current of very weak intensity, but if it was gradually increased the fish firstly reacts to it by (1) moving the upper barbels or fins, then, (2) swimming locomotion. Further increased intensity causes (3) electronarcosis, and finally (4) ends in death.

Table 1 shows the relation between the intensity of a current and the first reaction or movement of fins or barbels and the second reaction or swimming locomotion.

TABLE 1.

The first and second reactions of the catfish to electric current.

Number of animal	Body length in cm	Direction of current	First reaction: Movements of fins or barbels			Second reaction: Swimming locomotion		
			V	M.A	\bar{z}	V	M.A	\bar{z}
1	13.0	Ascending	10.39	1.86	0.078	17.60	3.21	0.134
		Descending	9.35	1.78	0.074	19.58	3.69	0.154
2	14.0	A	19.08	3.50	0.146	24.31	4.42	0.184
		D	16.41	3.16	0.131	26.10	5.02	0.209
3	15.2	A	10.22	1.97	0.082	19.44	3.74	0.156
		D	21.42	4.20	0.175	31.62	6.20	0.258
4	16.1	A	13.20	2.40	0.100	23.05	4.19	0.175
		D	14.96	2.77	0.120	19.44	3.60	0.150

Number of animal	Body length in cm.	Direction of current	First reaction Movements of fins or barbels			Second reaction Swimming locomotion		
			V	MA	δ	V	MA	δ
5	16.6	Ascending	8.99	1.65	0.069	16.36	3.00	0.125
		Descending	21.49	3.98	0.166	21.19	3.98	0.166
6	17.7	A	9.46	1.82	0.076	15.42	2.97	0.124
		D	12.16	2.25	0.091	13.88	2.57	0.107
7	17.8	A	9.21	1.71	0.071	20.84	3.86	0.161
		D	20.49	3.79	0.158	20.49	3.79	0.158
8	21.5	A	7.50	1.32	0.055	8.05	1.45	0.060
		D	7.43	1.40	0.058	10.35	1.95	0.081
9	30.2	A	10.00	2.00	0.083	25.65	5.13	0.214
		D	12.83	2.70	0.112	27.55	5.90	0.242
Average	18.01	A	10.90	2.03	0.084	18.97	3.55	0.148
		D	15.19	2.89	0.121	21.06	4.08	0.165

L body length of the fish V voltage between the electrodes (in volts)

MA. current intensity between the electrodes (in millampère)

δ current density per one millimetre square (in microampère)

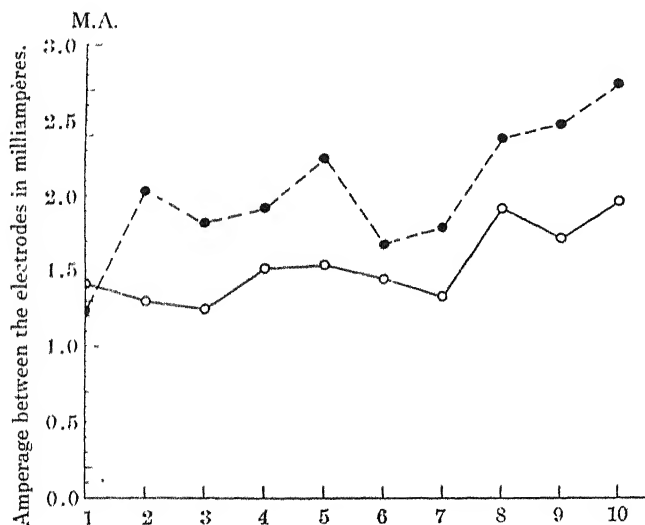


Fig. 1. Relation between current intensity and the repetition of tests

The order of trials

●...● L = 20.5 cm. --- Ascending current
○...○ L = 20.0 cm. — Descending current

In Table 1, it is seen that there is clear difference of current intensity between the first reaction and the second reaction. In the present experiment, I have paid my attention mainly to the second reaction or swimming locomotion.

In each experiment both the ascending and the descending currents were transmitted 5 or 10 times repeatedly on the same individual. In general the values of the current intensity vary requiring somewhat increasing intensity as the number of tests increased. For example, the behaviour of two catfish is shown in Fig. 1.

In the tables, I have, however, given only the mean values of 5 or 10 times of the experiments as the values for the second reaction.

2. Relation between the current intensity and the body length of the fish.

Relation of the electric current intensity of the body length of the fish is shown in Table II

TABLE II.

Relation of the electric current intensity to the body length of the fish.

Number of animals	Body length in cm. (l)	Direction of current	Temperature C°	Voltage between electrodes	V ₀	V ₀ /L	Current intensity between electrodes millampère	Current density per one mm 2 micro-ampère
5	12.5	Ascending	10.7	22.05	2.77	0.220	4.17	0.174
		Descending	10.9	24.17	2.89	0.238	4.67	0.194
5	15.7	A	9.4	18.98	2.94	0.190	3.67	0.145
		D	9.5	22.28	3.45	0.222	4.21	0.175
5	18.7	A	6.8	15.78	2.93	0.158	2.88	0.120
		D	7.0	15.76	2.94	0.158	2.88	0.120
5	20.2	A	5.9	10.69	2.16	0.107	1.78	0.074
		D	6.0	12.25	2.47	0.122	2.07	0.088
6	22.3	A	6.4	14.16	3.18	0.142	2.62	0.110
		D	6.6	18.64	4.16	0.186	3.49	0.145
7	23.5	A	8.4	17.74	5.08	0.177	3.24	0.144
		D	8.8	19.69	5.60	0.197	3.71	0.154
33	19.7	A	7.9	16.58	3.18	0.166	3.05	0.128
		D	8.1	18.80	3.59	0.187	3.51	0.146

V₀=potential drop per 1 cm. along the stream lines of the current in the aquarium without fish (in volts).

V₀/L=potential drop per 1 cm. along the fish body (in volts).

The values of an actual voltage that acts on the fish given by W. HOLZER (1931) is much less than the values calculated as V_0 from our own data.

For convenience the data given in Table II was divided into two groups, the smaller group, the fish which body length ranges from 11 cm. to 23 cm., and the larger group from 27 cm. to 30 cm.

The relation between the current density and the body length given by the two groups are shown in Fig. 2.

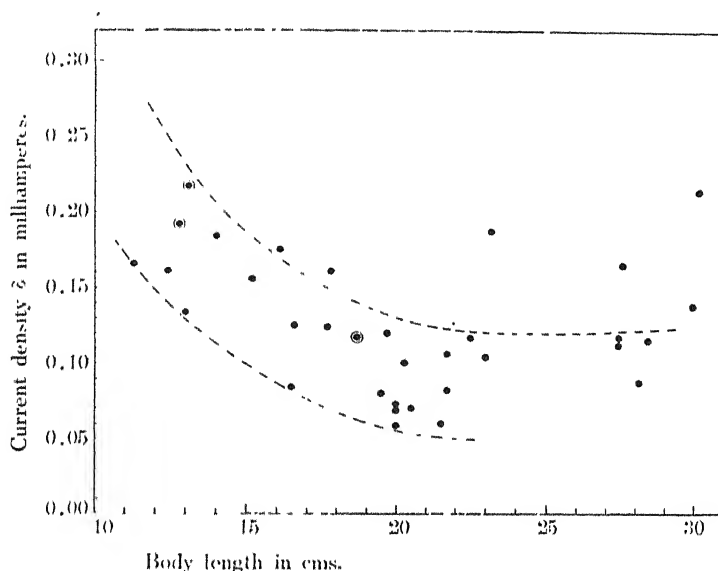


Fig. 2. Relation of the current density to the body length of the fish.

● showed locomotion. ● showed no locomotion

Fig. 2 shows clearly that the relation between the current intensity and the body length with the smaller group is approximately hyperbolic to 1 and ∂ axis; namely the smaller the individual the higher is its intensity in order to produce the locomotive reaction. In the larger group or beyond 23 cm. in body length such hyperbolic relation is not seen, indicating that weaker current enables the larger fish to move than that required by the smaller fish. The similar relation that is the relation of current density to the body length given in the above is seen when the relation between the values $V_0/\text{cm.}$ ($=V_0/L$) and the body length are plotted.

The fact that with the catfish the values of the current density differ with the different sizes of the body does not entirely agree with the values

shown by other kind of the fish tested by many other authors but I am unable, at present, to make any suggestion for such differences.

3. Directions of locomotion.

i) Individual characteristic of locomotion. The swimming reactions of the catfish by an electric current, may be divided into five types, (1) forward locomotion, (2) backward locomotion, (3) retreating locomotion, (4) right-ward locomotion and (5) left-ward locomotion.

The pass way of locomotion as well as the direction of locomotion more or less varies almost in all cases tested. We will show two typical examples of locomotion in Fig. 3.

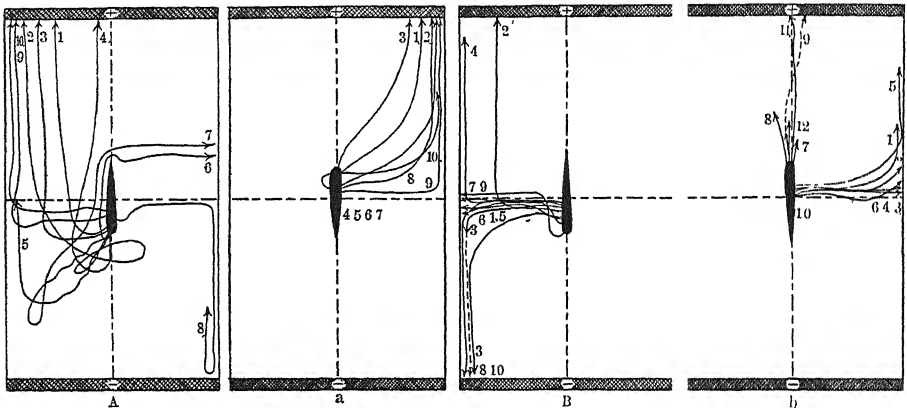


Fig 3. Direction of locomotion of catfish by electric current

A & a L=195 cm

A & B Ascending current.

B & b L=205 cm

a & b Descending current

In Fig. 3, it is seen clearly that the directions of locomotion as well as the pass ways taken by the catfish A are clearly different from those shown by B and b. Such differences shown by different catfish seem to us to be attributable to the manifestation of individual characteristics, and not to the difference of conditions of the experiment as the tests were made under nearly as identical conditions as possible.

ii) The directions of locomotion on galvanotaxis. The directions of locomotion by 33 catfish by an electric current are shown in Table III.

It will be noticed from the table that when the fish took the final direction 37% of trials were forward locomotion in the ascending current and 46% trials were backward locomotion indicating that the catfish did not show positive galvanotactic nature. In the case of the descending

TABLE III.

The directions of locomotion by an electric current.

Number of animal	L. in cm	Direction of the current	First direction					Final direction					Total cases	No of cases the fish showed no locomotion
			No. of cases the fish moved toward different direction											
			Foreward	Backward	Rightward	Leftward	Total cases	Foreward	Backward	Rightward	Leftward			
5	12.5	A	6	1	2	0	9	6	1	2	0	10	11	
		D	8	0	1	1	10	9	1	0	0	10	10	
5	15.7	A	12	4	7	5	28	16	11	0	1	28	4	
		D	10	4	5	2	21	13	7	1	0	21	9	
5	18.7	A	6	2	14	6	28	11	14	0	3	28	8	
		D	11	0	5	5	24	22	0	1	1	24	11	
5	20.2	A	5	5	28	5	43	15	18	10	0	43	9	
		D	6	0	21	4	34	8	10	16	0	34	15	
6	22.3	A	5	7	29	8	49	14	23	10	2	49	11	
		D	0	0	8	1	9	1	4	4	0	9	37	
7	28.5	A	11	9	15	6	41	11	25	5	0	41	4	
		D	4	3	6	6	19	12	7	0	0	19	25	
33	19.7	A	45	28	95	30	198	73	92	27	6	198	47	
		%	22.7%	14.1%	48.0%	15.2%		36.9%	46.5%	13.6%	3.0%		30.5%	
		D	42	7	43	19	111	65	29	22	1	117	107	
		%	37.8%	6.3%	38.7%	17.1%		55.6%	24.8%	18.8%	8.6%		69.5%	

current, however, 56% were foreward and only 25% were backward locomotion indicating in this case stronger tendency of positive galvanotaxis. In the first direction 14% of trials showed locomotion in the ascending current against 23% of foreward locomotion and in the descending current only 6% of trials showed the backward locomotion against 38% of foreward locomotion. From the above facts, it seems safe to consider that the catfish show a strong tendency towards foreward locomotion due probably to the results of combination in both foreward locomotion and the galvanotaxic nature. Namely the two factors are probably acting on the movement of fish in the following manner: in the case of an ascending current,

Positive galvanotaxic nature—foreward locomotion and in the descending current,

Positive galvanotaxic nature+foreward locomotion.

If the relation shown above were accepted, then it follows that the catfish behave in a positive galvanotaxic nature.

It will be seen also that the catfish under the action of the electric current show either the left-ward or the right-ward locomotion instead of either foreward and backward locomotion. When both the ascending and descending currents are combined the former showed 138 cases while the later showed 49 cases in their first direction and 19 cases and 7 cases in their final direction respectively. It seems worth noting that the cases of right-ward locomotion are far more frequent than in the cases of left-ward locomotion in both the first and final locomotion and also in the ascending current than in the descending current.

It is seen often that when a wooden rod is brought near to the head from the front side the fish shows retreating movement, but under the action of the electric current, I have seen but only once that the catfish retreated about 15 cm. when at the same time a knocking sound was made.

4. *The reaction of catfish in relation to the ascending and descending currents.*

The fact that the reactions of animals differ whether they were stimulated by the ascending current or by the descending current was already noted by J. LOEB and W. E. GARREY (1896) with *Amblystoma*. With the catfish the following facts were noted.

i) Current intensity. Intensity of an electric current that makes fish move differs according to the direction of the current. In general far stronger intensity is necessary in the case of a descending current than in the case of an ascending current. We have found only 5 exceptions to this general rule (Table II) out of 33 individuals employed. Averages taken from 33 individuals gave the following values:

TABLE IV.

No. of catfish used	Body length in cm. (l)	Direction of the current	Temp C.	Voltage between electrodes volt	Vo	Vo/L	Current intensity between electrodes milliampère	Current density per mm ² in micro-ampère
33	19.7	Ascending	7.9	16.58	3.18	0.166	3.05	0.128
		Descending	8.1	18.80	3.59	0.187	3.51	0.146

Vo=potential drop per 1 cm. along the stream lines of the current in the aquarium without fish (in volts).

Vo/L=Potential drop per 1 cm. along the fish body (in volts).

The fact that the different degree of intensity is required in order to produce the same kind of reactions, according to whether the catfish was stimulated ascendingly or descendingly was repeatedly demonstrated in our laboratory. NOMURA and ISHIKAWA (1933) found that 2.10 volts in ascending and 2.64 volts in descending were required in order to produce a convulsive movement when the catfish were tested in a very small aquarium of 30 mm. long, 5 cm. wide and 5.5 cm. deep.

Recently KOKUBO (1934) found that 3.8 volts in ascending and 19.5 volts in descending were required in order to produce "jumping reaction" of catfish if the fish was tested in a small aquarium of 23 cm. long 8 cm. wide and 12 cm. deep.

Owing to the different kind of reactions sought and different sizes of aquariums used, the values given above can not be directly compared, nevertheless all the results show that the same reactions are produced with less intensity of stimulus when transmitting the electric current ascendingly than transmitting it descendingly.

ii) Convulsive movements. It was noted that the catfish stimulated by the descending current show convulsive movements but no swimming locomotion. While, on the contrary, the ascending current causes swimming locomotion and very seldom (only two fish out of 33) shows convulsive movements. The cases which showed convulsive movement but no swimming locomotion were 107 in descending current and in an ascending current those which showed no swimming locomotion were 47 but those which showed convulsive movements were only 2 individuals as already stated.

iii) Reactions to knocking sounds. In Table III are shown many cases which showed no swimming locomotion by both ascending (47) and descending currents (107). To a sound of knocking on the edge of the aquarium by the end of a finger, these fish show quite different behaviour whether the fish were stimulated by the ascending current or by descending current. Nearly all the individuals which were stimulated by the ascending current begins to swim by a knocking sound. While those which were stimulated by a descending current fails to show swimming locomotion though some of them move the pectoral fins slightly.

The velocity of locomotion produced by a knocking sound appears to be faster than ordinary swimming locomotion produced by an electric current.

iv) Movements of fins. When the catfish is stimulated by gradually increased electric current, the dorsal fin is raised at first, and then the caudal fin is turned to either left-ward or right-ward. If the intensity of

the electric current is farther increased, that is about the intensity with which the fish show the convulsive movements, the caudal fin is raised by the descending current but is bent down by the ascending current as was already noted by LOEB on *Amblystoma*.

In general the pectoral fins are folded gradually in accompanying with the increased current intensity, but the cases were often found that the pectoral fins are folded by the ascending current and stretched by the descending current.

v) Manner of swimming locomotion. Whichever direction the fish faces at the beginning of the test when the fish swims from an anode to a cathode, usually presents a wavy or jumping locomotion while some suddenly shakes their head. All these actions appear to indicate painful sensation felt by the fish. On the other hand when the fish turns its direction of swimming from a cathode to an anode it swims comparatively in easy manner and the unnatural manner of locomotion mentioned above is no more to be seen.

The easiest manner of swimming is seen also when the fish turns its body vertically to the stream lines of the electric current. This is due probably since when the fish swims vertically to the stream lines, the voltage which acts on the fish is very much decreased than when the fish turns parallel to the stream lines.

GALVANOTAXIS OF THE CATFISH WITHOUT UPPER BARBELS

Normally the catfish stretches the upper barbels backwardly but if stimulated by the electric current the barbels are stretched forwardly before swimming locomotion starts. In order to test that if barbels were cut off, how the fish would react to the electric current, the following experiments using three fish of different body length were tried by the method already stated.

The directions of locomotion were not much modified by the absence of barbels though when the fishes became weaker the instances of no-locomotion appeared more frequently.

IV. GENERAL CONSIDERATIONS

1. E. BLASIUS and F. SCHWEIZER (1893) found that *Cyprinus carpio*, *Cobitis fossilis*, *Anguilla vulgaris* and 8 other species of fish showed positive galvanotropism. The same phenomenon is seen with the catfish, *Parasilurus asotus*, but at the same time the catfish show stronger nature of forward

locomotion, and in many cases the fish swam along the equipotential lines such as M. OKADA (1928) observed with the goldfish. This kind of locomotion lastly mentioned is considered to be identical with the so-called oscillotaxis by SCHEMINZKY (1924).

2. Already M. OKADA (1928), F. and F. SCHEMINZKY (1931), and W. HOLZER (1932) have found that there are constant relation between the current intensity and the body length of the fish, that is the smaller the fish, the higher is the values of V_0/L .

The relation just stated is also true with the catfish of smaller size (about 11 cm. to 23 cm.), but the relation is very much disturbed with individuals of larger size (about 27 cm. to 30 cm.) due probably from their sedentary behaviour.

3. On the manner of reactions of the fish to an electric current, F. and F. SCHEMINZKY (1931) say that "Schwache Ströme sind unwirksam; von einer gewissen Grenzstromstärke an wird die Stromschliessung durch eine Bewegung des ganzen Tieres, durch eine Zuckung oder dergleichen - je nach der Art des Tieres - markiert (erste Reaktion), bei stärkeren Strömen tritt während der Stromflusszeit eine gegen die Elektroden gerichtete Bewegung auf (Elektrotaxis), bei noch stärkeren eine Lahmung (Elektronarkose)." Such four kinds of reaction are also seen in the catfish.

SCHEMINZKY have found the ratio of the values of a current density between "erste Reaktion" and "Elektrotaxis" is 1 : 1.5 in *Phoxinus laevis*, and 1 : 6.5 in *Cottus gobio*. We found with *Parasilurus asotus* that the ratio was 1 : 1.45 with the descending current and 1 : 1.77 with the ascending current.

4. In Table V various values taken from several species of fishes are compared with our own data taken from the catfish.

In Table V, it is seen that *Parasilurus asotus* gives higher values of V_0/L than that given by *Cyprinus carpio* and goldfish to such an extent that the former gives about 4 to 7 times higher than in the later in the first reactions. Although the catfish shows about the same values of current density as *Phoxinus laevis* and *Cottus gobio* for the first reactions but for the galvanotaxis, the former differs conspicuously from the latter. The reason for such a great difference is difficult to explain. Perhaps the difference may be due to the differences of methods used rather than the differences of species or of their body length.

5. The intensity of currents used for producing the swimming locomotion is too strong when compared with the intensity of the earth current ;

TABLE V.
The reactions of fish to electric current.

Species name	L in cm.	First reaction		Galvanotaxis		
		Vo/L in Millivolts	in L A	Vo/L in Millivolts	in L A	
Cyprinus carpio 1	0.6-11.0	13-50				
Goldfish 2	1.0-12.0	27-60				
Phoxinus laevis 4	1.5- 7.5		0.13-0.19		1.28-3.30	
Cottus gobio 5	6.0- 8.7		0.14-0.18		0.89-1.26	
Parasilurus asotus 3	13.0-30.2	90-205	0.069-0.158	85-256	0.059-0.214	A
		94-214	0.074-0.175	71-316	0.063-0.258	D

(1) and (2) after M. OKADA

(4) and (5) after F. and F. SCHEMINZKY

A .ascending current. D .descending current

the value which may seldom exceed 100 millivolts per 50 metres. But it will be interesting to cite here a few examples of experiment which show an occurrence of galvanotaxis with the minimum intensity of the electric current.

Body length.	V	Vo/L	M.A.	δ
20.0 cm.	6.60	0.066	1.10	0.046
20.5 cm.	4.13	0.041	0.78	0.033

In this connection we wish to emphasize the following facts strongly: that is the purpose of the present experiment was to determine the threshold values of electrical intensity in order to induce swimming movement to the catfish forcibly and not the observation of swimming by their own will. Indeed the catfish do swim without any artificial stimulus applied or in some cases the catfish stubbornly refuse to swim even when many times stronger electrical stimulus than the values given in the present report applied (See also Table III for number of cases the catfish do not show any movement). Therefore the fact that the catfish needed far more intense stimulus in order to induce the movement than that of the natural earth currents, can not be taken literally as meaning that the fish can not react to the weaker electrical stimulus such as the values given by the earth-current. Quite contrary, as several authors have already found the catfish is perhaps one of the most sensitive fish and responds to as small current as one microampère. PARKER (1917) and UZUKA (1934) demon-

strated that the electrolytes dissolved from the surface of thin metal rods are sufficiently stimulative to induce the catfish to the movement of barbels, and fins and even the entire body either towards or away from the rods. KOKUBO (1934) noticed the catfish show "jumping reaction" with as small quantity of electricity as 0.0125 microampere per square mm. which means that the catfish can jump by nearly the same order of magnitude of electrical current as with the natural earth currents.

SUMMARY

1. The catfish, *Parasilurus asotus* exhibits four different kinds of reactions in succession to gradually increasing electric currents: (1) the movement of upper barbels or fins, (2) swimming locomotion, (3) electro-narcosis and finally (4) death.

2. When the catfish is stimulated by the electric current repeatedly in short intervals, the values for producing the galvanotaxic reaction tend to increase with the repetition.

3. The shorter the body length, the higher is the values of V_0/L , but beyond certain length, this relation is not clearly shown due probably to the sedentary life of older catfish.

4. Essentially the catfish show positive galvanotaxis, but there are many individuals that swim along the equipotential lines.

5. (i) In order to produce the same kind of reactions much higher values of current intensity is required when the fish is stimulated in an ascending direction than in a descending direction.

(ii) The cases of no-locomotion are far more frequent when the current was passed descendingly than ascendingly. The convulsive movements occur more frequently in association with the descending current.

(iii) Nearly all of the fishes showed swimming locomotion by a knocking while transmitting the current ascendingly but rarely the descending current.

(iv) The caudal fin is uplifted by the ascending current and bent down by the descending current. Though the pectoral fins are folded usually by both ascending and descending currents some fold the fins in the ascending current and stretch it in the descending current.

(v) The swimming of catfish toward an anode appears more natural than toward a cathod and still more natural when swimming along the equipotential lines.

REFERENCES

- BLASIUS, E. und SCHWEIZER, F. 1893. Elektrotropismus und verwandte Erscheinungen. Pflügers Arch., Bd. 53, PP. 193-543.
- HATAI, SHINKISHI and ABE, NOBORU 1932. The response of the catfish, *Parasilurus asotus*, to earthquakes. Proc. Imperial Acad., Vol. VIII, No. 8, PP. 375-378.
- HATAI, SHINKISHI, KOKUBO, SEIJI and ABE, NOBORU 1932. The earth currents in relation to the responses to catfish. Proc. Imperial Acad., Vol. VIII, No. 10, PP. 478-481.
- HOLZER, WOLFGANG 1932. Über eine absolute Reizspannung bei Fischen. Pflügers Arch., Bd. 229, PP. 153-172.
- KOKUBO, S. 1934. On the Behaviour of Catfish in Response to Galvanic Stimuli. Sci. Rep. Tōhoku Imp. Univ. Biol., Vol. IX, No. 2.
- LOEB, J. and GARREY, W. E. 1896. Zur Theorie des Galvanotropismus. II. Versuche an Wirbeltieren. Pflügers Arch., Bd. 65, PP. 41-47. (cited from J. LOEB, 1918. Forced movements, tropisms, and animal conduct. PP. 40-41).
- NOMURA, S. and ISHIKAWA, K. 1933. Response of Fishes to the Change of Environmental Factors. II. Preliminary Experiments in the Measurement of Chronaxie in Fishes. Annual Rep., Saitō Ho-On Kai, No. 9, Dec.
- OKADA, MITSUYO 1929. On the reaction of electric current on fishes I. Excitation and narcosis. Jour. Imperial Fisheries Institute, Vol. XXIV, No. 2, PP. 64-72.
- PARKER, G. H. and HEUSEN, A. P. 1917. The responses of the catfish, *Amiurus nebulosus*, to metallic and non-metallic rods. Am. Jour. Psychol., Vol. 44, No. 3.
- SCHEMINZKY, FERDINAND 1924. Versuche über Elektrotaxis und Elektonarkose. Pflüger's Arch., Bd. 202, PP. 200-216.
- SCHEMINZKY, FERDINAND und SCHEMINZKY, FRIEDRIKE 1931. Körpergrösse und Empfindlichkeit gegen den galvanischen Strom. Ibid., Bd. 228, PP. 548-564.
- UZUKA, KIYOSHI 1934. Some notes on the behaviour of the catfish, *Parasilurus asotus*, as seen through the responses to weak electric current. Sci. Rep. Tōhoku Imp. Univ. Biol., Vol. VIII, No. 4, PP. 369-381.

CHEMICAL COMPOSITION OF THE BODY FLUID OF AN ASCIDIAN: *CHELYOSOMA SIBOJA* OKA¹⁾

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In my previous paper (1933), the author showed that the body fluid obtained from a cut of the test of an ascidian, *Chelyosoma siboja* OKA, living in Mutsu-Bay presents a high acidic reaction due to the presence of free sulfuric acid. The acid was demonstrated chiefly by the determination of the anions, related to acid and by the electrometric titration of the original body fluid, plasma and the corpuscle fluid. The concentration of the acidity was found to be 4.3% (pH 0.38) in the corpuscle, 1.8% in the body fluid and 0.13% (pH 1.80) in the plasma respectively. The ratios of SO_4/Cl were estimated to be 20.14 (freezed) or 333.7 (diluted) in the corpuscle fluid, 1.86 in the body fluid and 0.231 in the plasma, as in contrast to 0.1158 in the sea water. It was further found that the values of the osmotic pressure is almost the same as the surrounding sea water.

These results obtained from our ascidian differed from that obtained by HENZE (1911, 1912) from the blood of the other ascidian, *Phallusia mammillata*, found in the Mediterranean.

In regard to the inorganic composition of *Phallusia mammillata*, HENZE (1912) gave the following results.

Chemical composition of the blood of *Phallusia mammillata*.

Gram in 100 cc.

	Plasma	Corpuscle	Naples sea water
K ₂ O	0.0562	—	0.0514
CaO	0.0651	—	0.0660
MgO	0.2299	—	0.2322
Cl	2.2612	—	2.1732
SO ₄	0.1297	—	0.2546
SO ₄ /Cl	0.0558	2.55	0.1171
Σ	2.14		2.20

¹⁾ Contribution from the Marine Biological Station, Asamushi, Aomori-ken. No. 119.

²⁾ I am greatly indebted to Prof. S. HATAI in reading and criticizing the manuscript.

As the table shows, HENZE performed neither the sodium determination in the plasma nor on the chemical composition of the corpuscle fluid, but merely showed a relative concentration of SO_4/Cl to be 2.55.

In this paper, the present author attempts to determine the distribution of the ions both in the plasma and in the corpuscle of the ascidian, *Chelyosoma siboja* OKA which was markedly different from the sea water. Special attention was given to the distribution of the ions within and without the cell in the corpuscle cell at the high concentration of sulfuric acid. This ascidian gave special advantage for the purpose of this study because of the large quantity of the corpuscles which could be obtained in such quantity as it reaches 40–30% in volume together with the large quantity of the cell sap. This work was undertaken partly at The Asamushi Marine Biological Station and partly at The Biological Institute in Sendai.

METHOD AND TECHNIQUE

The fresh ascidia were collected during the month of July from the sea, and cleaned of the foreign objects attached to the body surface. Then the animal was cut at the ridge on the posterior end of the test near the exhalent syphon and the body fluid containing the corpuscle was gently squeezed out. The body fluid thus collected yielded about 800 c.c. from 60 individuals. The volume of the corpuscle was measured by the centrifugarization for 20 minutes at 3000 r.p.m.

The chemical analysis was carried out on the body fluid, the plasma and the corpuscle fluid. The first 50 c.c. of the body fluid was diluted 10-fold in the volumetric flask. The remaining body fluid was centrifugarized, then the supernatant fluid thus separated was used for analysis of the plasma fluid. After sucking up the plasma the corpuscle was obtained from the sediment by again centrifugarizing for 30 minutes or more, in order to remove as much as possible the plasma fluid which adhered to the corpuscle surface.

The washing of the corpuscle can not be performed with the isotonic salt solution as is usually applied in order to avoid the diffusion of the acid from the corpuscle into the plasma.

One part of the corpuscle mass thus obtained was diluted ten times with distilled water in the volumetric flask while the another part of the corpuscles was frozen by the cooling mixture of ice and common salt in stirring often with the glass rod. The freezing process was repeated three times by melting each time placing the bottle in warm water. This

process produces complete plasmolysis of the corpuscle cell and the stroma was removed by centrifugarizing. The sea water used for comparison was collected at the same place where the materials were found. The analyses were carried out by the following methods, sodium by the method of KRAMER and GITTLEMAN, potassium for KRAMER and TISDALL. Calcium by CLARK-COLIP modification of the KRAMER and TISDALL method, magnesium by DENIS method, chlorine by WHITEHORN or VOLHARD method and inorganic sulphate by KAIIN and LEIBOFF method. The benzidin sulphate was titrated volumetrically with 0.02 N. NaOH instead of by the colorimetric method.

Total acidity was determined by the titration with 0.1 N. NaOH by phenolphthalein.

The protein in the ascidian fluid was removed by precipitating with tungstic acid by the method of FOLIN and WU or by the treating with 20% trichloroacetic acid.

EXPERIMENTAL RESULTS

In this experiment, the volume of the corpuscle contained in the ascidian body fluid was 30%, while fluid content of the corpuscle itself was 90%, the remaining 10% being represented by the sedimented stroma.

The freezing point depression was 1.85 in the plasma and 2.05 in the

TABLE 1.
Inorganic composition of ascidian fluids and of Asamushi sea water.
Gram in 1000 c.c.

	Asamushi sea water	Ascidian fluid			
		Plasma	Body fluid	Corpuscle (diluted)	Corpuscle (frozen)
Cl	19.36	17.38	12.62	2.66	2.30
Na	10.90	10.48	7.69	1.22	1.25
K	0.40	0.53	0.70	1.68	2.30
Ca	0.425	0.465	0.557	0.738	* 0.061
Mg	1.33	1.01	0.73	0.15	0.15
SO ₄	2.71	4.74	18.04	41.65	43.17
H	—	0.034 N.	0.267 N.	0.711 N	0.738 N.
Δ	1.95	1.85	—	—	2.05

* The amount of the frozen corpuscle-fluid is less than that found in the ten-times diluted solution of the corpuscle. Less amount of calcium here found may be due to the presence of sulfuric acid, forming a precipitation of calcium sulfate.

corpuscle fluid, while that of the Asamushi sea water was at 1.95.

The analyses of the ascidia together with that of the sea water are shown in Table 1.

As will be seen from the above table, comparing, the SO_4 content of ascidian fluids in all cases is always greater than the acidity calculated from the normality titrated by the standard alkali. The difference between the inorganic SO_4 and acid SO_4 contained in sulfuric acid may represent the amount of non-acid SO_4 . The relation just stated is shown in Table 2.

TABLE 2.

	Plasma	Body fluid	Corpuscle (diluted)	Corpuscle (frozen)
Inorganic SO_4	4.74	18.04	41.65	43.17
Acid SO_4	0.034 N.=1.63	0.267 N.=12.83	0.711 N.=34.15	0.738 N.=35.45
Non-acid SO_4	3.11	5.21	7.50	7.72

As will be seen in Table 2, SO_4 in the ascidian fluid exists in two states, which behave as non-acid SO_4 and as the acid SO_4 . Non-acid SO_4 is less concentrated than that of acid- SO_4 . The amount of the non-acid SO_4 which was found in the ascidian is always greater than that found in the sea water.

The value of SO_4 and the values of other inorganic components given

TABLE 3.

*Composition of ascidian fluids and Asamushi sea water
expressed as gram ion.*

	Asamushi sea water	Ascidian fluid			
		Plasma	Body fluid	Corpuscle (diluted)	Corpuscle (frozen)
Cl^-	0.546	0.490	0.356	0.075	0.065
Na^+	0.474	0.456	0.334	0.053	0.054
K^+	0.010	0.014	0.018	0.043	0.059
Ca^{++}	0.011	0.012	0.014	0.018	0.002
Mg^{++}	0.055	0.042	0.030	0.006	0.006
SO_4^{--}	0.028	0.032	0.054	0.078	0.080
Acid- SO_4^{--}	—	0.017	0.134	0.356	0.369
H^+	—	0.034	0.267	0.711	0.738
Total	1.124	1.097	1.207	1.340	1.373

TABLE 4.

Gram ionic proportion of ascidian fluids and Asamushi sea water expressed as percentage of Cl.

	Asamushi sea water	Ascidian fluids			
		Plasma	Body fluid	Corpuscle (diluted)	Corpuscle (frozen)
Cl'	100.00	100.00	100.00	100.00	100.00
Na'	86.81	93.06	93.82	70.67	83.08
K'	1.83	2.86	5.06	57.33	90.77
Ca''	2.02	2.45	3.93	24.00	3.08
Mg''	10.07	8.57	8.43	8.00	9.23
SO ₄ ''	5.13	6.53	15.17	104.00	123.08
Acid-SO ₄ ''	—	3.47	37.61	474.67	567.69
H'	—	6.91	75.00	948.00	1135.38

in Table 1 are compared with those of the sea water with respect to the gram ionic concentration as well as the gram ionic proportion which is expressed as percentage of Cl in Table 3 and 4.

GENERAL REMARKS

The amount of sodium, magnesium and chlorine in sea water are nearly identical with that found in the plasma, but are very considerably less in the corpuscle fluid, though the amount of potassium and that of calcium are much concentrated. The amount of acid SO₄ in the corpuscle, is absolutely greater than that in the other fluids and sea water. Furthermore the amount of the non-acid SO₄ in the corpuscle is 2.77 (diluted) or 2.86 (frozen) times greater than in sea water. It is also evident, as will be seen from Table 3 and 4, that a greater fraction of SO₄ in the corpuscle exists in the form of sulfuric acid. The sulfuric acid in the corpuscle fluid may serve in maintaining the osmotic pressure similar to the osmotic pressure of sea water and of plasma which is regulated chiefly by the NaCl.

The general feature of the distribution of the various ions in the plasma is also different from sea water in such a way that sodium, magnesium and chlorine are slightly less but potassium, calcium and both acid and non-acid SO₄ are greater in the plasma than in the sea water.

The amount of various ions in the body fluid, stands between those found in the plasma and in the corpuscle fluid. The acidity determined in the present work, gives lower value, eg. 0.738 N. in the corpuscle fluid,

and also 0.267 N. in the body fluid, than that given in the previous work (0.88 N. in the corpuscle fluid, 0.360 in the body fluid). On the contrary the acidity given by the plasma (0.034 N) is higher than that found in the previous work (0.027 N.).

One of the sources of such difference might be due to the diffusion of acid from the corpuscle cell into the plasma, while collecting larger quantity of the sample fluid for analysis.

The results obtained in this experiment, differ somewhat from those obtained by HENZE who, however, analysed the plasma of *Phallusia mammillata*, but not the corpuscle.

The data obtained by HENZE were calculated from his published results for the purpose of direct comparison with the present experiment and are shown in Table 5.

TABLE 5.

	Gram in 1000 c.c		Gram ionic concentration		Gram ionic proportion	
	Naples sea water	Plasma	Naples sea water	Plasma	Naples sea water	Plasma
Cl'	21.732	22.612	0.613	0.638	100.00	100.00
Na'	—	—	—	—	—	—
K'	0.427	0.467	0.011	0.012	1.79	1.88
Ca''	0.472	0.465	0.012	0.012	1.96	1.88
Mg''	1.400	1.390	0.058	0.057	9.40	8.97
SO ₄ ''	3.055	1.560	0.032	0.016	5.22	2.51
Δ	2.20	2.14				

The amount of chlorine found by HENZE from the plasma is higher than that found in *Chelyosoma siboga*, but less in non-acid SO₄ and Ca. On the other hand, acid SO₄ was absent in HENZE's analysis of *Phallusia mammillata*.

SUMMARY

It is evident from the above experiment that free sulfuric acid is accumulated in a larger quantity in the corpuscle than Na, Mg and Cl, in spite of the facts that Na and Cl are greatly predominated in the liquid media, sea water and plasma, which bathe the corpuscle cells. In this connection, it may be noted that non-acid SO₄ is found to be relatively highly concentrated as also with K and Ca.

The plasma of *Chelyosoma siboga* appears to be different from that of the blood of *Phallusia mammillata* as the later lacks acid SO_4 and less concentration of non-acid SO_4 and Ca in the plasma. Whether or not this difference may partly be due to the presence or absence of the different kinds of corpuscle cells in the body fluid of the two kinds of ascidian used by HENZE and by the present author remain to be tested in the future.

REFERENCE

1. CLARK, E. P. and COLLIP, J. B. 1925. A study of the TISDALL method for the determination of blood serum calcium with a suggested modification. Jour. Biol. Chem., Vol 63, pp 461-461.
2. DENIS, W. 1922 The determination of magnesium in blood plasma and serum *ibid*, Vol 52, pp 411-415
3. FOLIN, O. and WU, H. 1919 A system of blood analysis. *ibid*, Vol 38, pp. 80-110.
4. HENZE, M. 1911. Untersuchungen über das Blut der Ascidien. I Zeitsch. f. physiol. Chem, Bd 72, pp 494-501
5. HENZE, M. 1912. Untersuchungen über das Blut der Ascidien. II *ibid*, Bd 79, pp. 215-228
6. KAHN, B. S. and LEIBOFF, S. L. 1928. Colorimetric determination of inorganic sulphate in small amount of urine. Jour. Biol. Chem, Vol. 80, pp 623-629.
7. KOBAYASHI, S. 1933 Studies on acid of the body fluid from ascidian, *Chelyosoma siboga* OKA. Sci. Rep. Tōhoku Imp Univ Biol, Vol. 8, pp. 277-285.
8. KRAMER, B. and GITTLEMAN, I. 1924 An iodometric method for the determination of sodium in small amount of serum. Jour. Biol. Chem, Vol 62, 353-360.
9. KRAMER, B. and TISDALL, F. 1921 A clinical method for the quantitative determination of potassium in small amount of serum. *ibid.*, Vol. 46, pp 339-349.
10. WHITEHORN, J. C. 1921. A system of blood analysis. *ibid*, Vol. 45, pp 449-460.

ON THE SURVIVAL POWERS OF MALES AND FEMALES OF *CARASSIUS AURATUS* (L.) UNDER SOME HARMFUL EXTERNAL CONDITION

By

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(With three figures)

(Received November 5, 1934)

INTRODUCTION

It is a well-known fact that the sex-ratio varies considerably according not only to the species but even in the same species under different conditions such as season, inanition etc. *Carassius auratus* (L.) is also one in which the number of females far exceeds that of the male. That by what factors the sex-ratio is determined should be studied from various angles, as for instance, differential death rate, sex inversion etc. I have at first attempted to determine whether or not a differential death rate in the juvenile and adult period was chiefly responsible, and the results of the experiment are shown in this present report.

The writer offers his sincerest gratitude for the kind instruction and encouragement given in this work by Dr. S. HATAI.

MATERIAL AND METHOD

According to BADE ('23) the silver carp, *Carassius vulgaris* NILS, lives in central Europe and central Asia but the silver carp in Japan belongs to *Carassius auratus* (L.) and is extremely common in rivers, ponds, marshes, lakes, and also brackish water. The silver carp is easily distinguishable from the generally known common carp (*Cyprinus carpio* L.) by its lack of barbels, the number of rays in the dorsal (20 rays), and an anal fin (9 rays).

The body of the silver carp exhibits olive colour except for the ventral part which is white in colour. The length of body reaches sometimes as long as 30 cm. According to SASAKI ('26) the silver carp lay eggs several times during the months from May to July.

Carassius auratus used in the present experiment are those obtained from Oedo-bori, a small creek about 1.5 m. wide and 0.5 m. deep, in

Harano-machi, Sendai, caught at random with a fine net. The fishes thus obtained were kept for 2-3 days before use in an aquarium. For the experiment the fishes of over two month old were used.

I have tested the relative survival power keeping the fish under O_2 -deficiency condition and also under various concentrations of NaCl-solution. The fishes were kept in a glass jar of 1 litre volume which was filled with tap-water. To make O_2 -deficiency condition the surface of water was covered 1 cm. thick with liquid paraffin. During the course of the experiment the fishes were not fed at all but I may mention that *Carassius auratus* can survive for a considerably longer period without food.

The sexes, if the fishes were over two month old, can be distinguished by the colour of their gonads even with the naked eye; the gonad of a female in an early stage is transparent, for somewhat matured stage light yellow and in the wholly matured stage orange yellow together with a granular appearance. On the contrary in the case of males their gonads are milky white at all stages of maturation.

RESULT

Survival power of males and females of Carassius auratus in the water.

1) 5, 10, 15, 20 young *Carassius* (4-7 cm. in total length) were kept separately in four glass jars filled with 4 litres of tap-water. The water was covered 1 cm. thick with liquid paraffin. The same experiment was repeated several times. The results of the experiment are shown in Tables 1-4.

TABLE 1.

5 fishes in each jar filled with water, the surface of which is covered with liquid paraffin.

	Hours	20-40	40-60	60-80	80-100	100-120	120-140	Total
	Sex							
Number of fishes	♀	25	14	11	2	1	6	59
	♂	0	2	0	2	0	3	7
Totals of survival hour	♀	750	700	770	180	110	780	3290
	♂	0	100	0	180	0	390	670
Survival hour per fish	♀							55.7
	♂							95.7

In this table and also in others, the fishes whose survival hours are uncertain, were omitted.

TABLE 2.

10 fishes in each jar filled with water, the surface of which is covered with liquid paraffin.

	Hours	15-20	20-25	25-30	30-35	35-40	Total
	Sex						
Number of fishes	♀	6	7	0	1	1	15
	♂	4	6	0	1	0	11
Totals of survival hour	♀	105	157.5	0	32.5	37.5	332.5
	♂	70	135	0	32.5	0	237.5
Survival hour per fish	♀						22.2
	♂						21.5

TABLE 3.

15 fishes in each jar filled with water, the surface of which is covered with liquid paraffin.

	Hours	1-3	3-5	5-7	7-9	9-11	11-13	Total
	Sex							
Number of fishes	♀	2	6	61	13	11	0	96
	♂	0	1	3	2	1	1	8
Totals of survival hour	♀	4	24	384	104	110	0	626
	♂	0	4	18	16	10	12	60
Survival hour per fish	♀							6.5
	♂							7.5

TABLE 4.

20 fishes in each jar filled with water, the surface of which is covered with liquid paraffin.

	Hours	1-3	3-5	5-7	7-9	9-11	11-13	13-15	15-17	17-19	19-21	21-23	23-25	25-27	27-29	Total
	Sex															
Number of fishes	♀	3	95	79	30	3		3	3	1	1	1				219
	♂	0	7	14	6	1		0	3	0	0	0				31
Totals of survival hour	♀	6	380	474	240	30		60	66	24	26	28				1334
	♂	0	28	84	48	10		0	66	0	0	0				236
Survival hour per fish	♀															6.09
	♂															7.6

2) 3, 5, 10, 15, 20 adult *Carassius* were kept separately in each jar

which was filled with 4 litres tap-water covered 1 cm. thick with liquid paraffin. The results are shown in Tables 5-9.

TABLE 5.

3 fishes in each jar filled with water, the surface of which is covered with liquid paraffin.

	Hours Sex	10-15	15-20	20-25	25-30	30-35	35-40	40-45	Total
Number of fishes	♀	36	77	43	13	0	3	0	172
	♂	8	23	13	12	1	7	2	66
Totals of survival hour	♀	450	1347.5	967.5	357.5	0	112.5	0	3235
	♂	100	402.5	292.5	330	32.5	262.5	85	1505
Survival hour per fish	♀								18.8
	♂								22.8

TABLE 6.

5 fishes in each jar filled with water, the surface of which is covered with liquid paraffin.

	Hours Sex	5-10	10-15	15-20	20-25	25-30	30-35	35-40	Total
Number of fishes	♀	13	7	17	27	9	1	3	77
	♂	0	0	7	5	0	0	0	12
Totals of survival hour	♀	97.5	87.5	297.5	607.5	247.5	32.5	112.5	1482.5
	♂	0	0	122.5	112.5	0	0	0	235
Survival hour per fish	♀								19.25
	♂								19.58

TABLE 7.

10 fishes in each jar filled with water, the surface of which is covered with liquid paraffin.

	Hours Sex	2-5	5-8	8-11	..	17-20	20-23	23-26	Total
Number of fishes	♀	10	12	3		1	7	9	42
	♂	0	0	2		0	0	0	2
Totals of survival hour	♀	35	78	28.5		18.5	150.5	220.5	531
	♂	0	0	19		0	0	0	19
Survival hour per fish	♀								12.6
	♂								9.5

TABLE 8.

15 fishes in each jar filled with water, the surface of which is covered with liquid paraffin.

	Hours	3-5	5-7	7-9	9-11	Total
	Sex					
Number of fishes	♀	57	33	11	2	103
	♂	2	0	0	0	2
Totals of survival hour	♀	228	198	88	20	534
	♂	8	0	0	0	8
Survival hour per fish	♀					5.18
	♂					4.0

TABLE 9.

20 fishes in each jar filled with water, the surface of which is covered with liquid paraffin.

	Hours	1-3	3-5	5-7	7-9	9-11	Total
	Sex						
Number of fishes	♀	17	63	31	3	1	115
	♂	0	1	2	0	0	3
Totals of survival hour	♀	34	252	186	24	10	506
	♂	0	4	12	0	0	16
Survival hour per fish	♀						4.4
	♂						5.3

The survival hour per fish in each test above given is shown graphically in Figure 1 (Based on Tables 1-9).

Survival power of males and females of Carassius auratus in the sodium chloride solution.

1) Young *Carassius*. 10 fishes were kept in each jar filled with 4 litres of tap-water in NaCl-solution of various concentrations. The tap-water was used as a solvent and the concentrations were equivalent to 0.3 mol, 0.25 mol, and 0.2 mol were tested and the results of the experiment are shown in Tables 10-12.

TABLE 10.

10 fishes in each jar filled with 0.3 mol solution of NaCl.

	Hours	0-1	1-2	2-3	Total
	Sex				
Number of fishes	♀	4	71	15	90
	♂	0	6	4	10
Totals of survival hour	♀	2	106.5	37.5	146
	♂	0	9	10	19
Survival hour per fish	♀				1.62
	♂				1.90

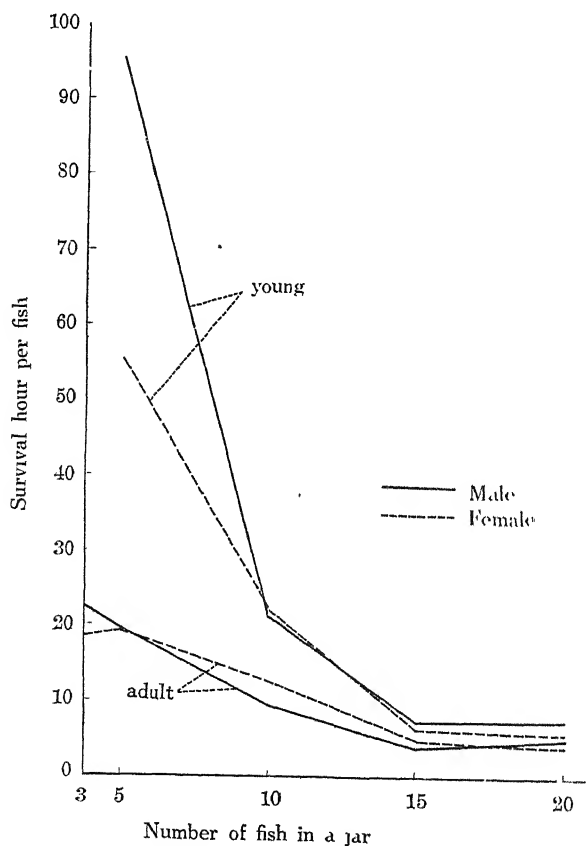


Fig. 1. Showing the survival power of male and female fishes kept in tap-water covered with liquid paraffin

TABLE 11.

10 fishes in each jar filled with 0.25 mol solution of NaCl.

	Hours	1-5	5-9	9-13	13-17	17-21	21-25	25-29	Total
	Sex								
Number of fishes	♀	59	59	2	0	2	3	1	126
	♂	1	9	1	0	1	2	0	17
Totals of survival hour	♀	177	113	22	0	38	69	27	746
	♂	12	63	11	0	19	46	0	151
Survival hour per fish	♀								5.92
	♂								8.88

TABLE 12.

10 fishes in each jar filled with 0.2 mol solution of NaCl.

	Hours	20-40	40-60	60-80	80-100	100-120	120-140	Total
	Sex							
Number of fishes	♀	30	8	15	32	0	27	112
	♂	1	0	4	20	0	7	32
Totals of survival hour	♀	900	100	1050	2880	0	3510	8740
	♂	30	0	280	1800	0	910	3020
Survival hour per fish	♀							78.03
	♂							94.37

2) Adult *Carassius*. 10 fishes were kept in each jar filled with 4 litres of tap-water in NaCl-solution of various concentrations. The tap-water was used as a solvent and the concentrations were equivalent to 0.32 mol, 0.3 mol, and 0.25 mol were tested and the results of the experiment are shown in Tables 13-15.

TABLE 13.

10 fishes in each jar filled with 0.32 mol solution of NaCl.

	Hours	0-1.5	1.5-3.0	3.0-4.5	Total
	Sex				
Number of fishes	♀	33	60	2	95
	♂	1	4	0	5
Totals of survival hour	♀	24.75	135.0	7.5	167.25
	♂	0.75	9.0	0	9.75
Survival hour per fish	♀				1.75
	♂				1.95

TABLE 14.
10 fishes in each jar filled with 0.3 mol solution of NaCl

	Hours	0-2	2-4	4-6	6-8	8-10	10-12	Total
	Sex							
Number of fishes	♀	19	103	56	33	11	4	226
	♂	3	19	5	2	0	2	31
Totals of survival hour	♀	19	309	280	231	99	41	982
	♂	3	57	25	14	0	22	121
Survival hour per fish	♀							4.34
	♂							3.90

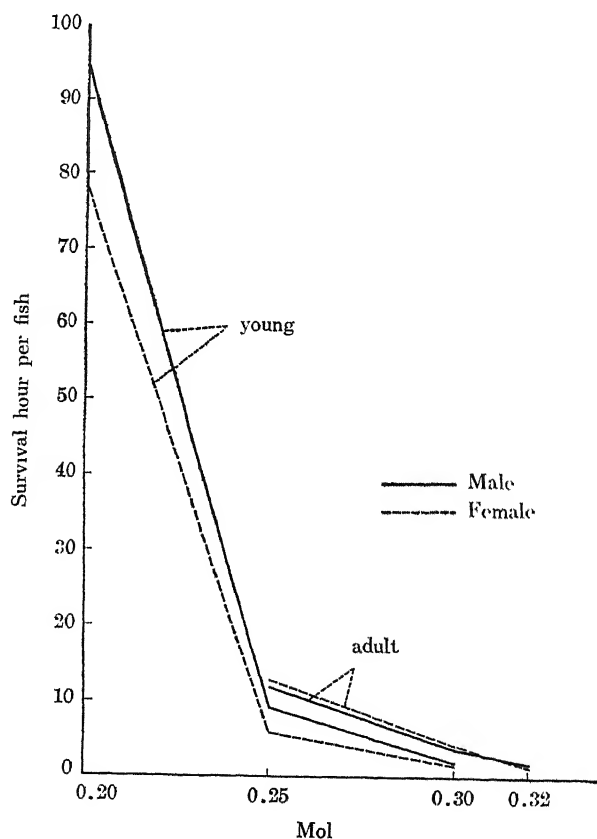


Fig. 2. Showing the survival power of male and female fishes kept in NaCl-solution of various concentrations.

TABLE 15.

10 fishes in each jar filled with 0.25 mol solution of NaCl

		Hours													
Sex		0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	Total		
Number of fishes	♂	38	63	13	1	15	10	1	1	0	1	1	174		
	♀	7	20	5	2	8	1	2	0	0	0	0	45		
Totals of survival hour	♂	95	172.5	162.5	17.5	1012.5	275	32.5	37.5	0	47.5	52.5	2205		
	♀	17.5	150	62.5	35	180	27.5	65	0	0	0	0	537.5		
Survival hour per fish	♂												12.6		
	♀												11.9		

The survival power per fish in NaCl-solution given above is shown in Figure 2 (Based on Tables 10-15).

Carassius auratus and its sex-ratio.

SASAKI (26) who examined the sex-ratio in *Carassius auratus* from extensive materials has shown that the sex-ratio varies regularly with the sizes of the fishes

2182 fishes collected by the present writer at random at Harano-machi, Sendai (April-December 1933) gave the sex-ratio 16.55 ♂ : 100 ♀, while 1188 fishes collected in Saga, Kyushu during October 1933 gave the sex-ratio 36.86 ♂ : 100 ♀.

In the later all fish in one pond were collected and the relation between the sex-ratio and body length was as follows:

Body length in cm.	No. of ♂ to each 100 ♀.	Actual No.	
		♂	♀
4 to 7	45.8	271	591
7 to 10	21.3	32	150
10 to 15	20.4	17	83
15 -	0	0	44

The relation presently found generally agrees with that found by SASAKI; that is the number of males per 100 females diminishes progressively with the increasing size of the fishes.

Ova-containing testis in Carassius auratus.

The gonads of the male of *Carassius auratus* as was already mentioned, exhibit milky white coloration irrespective of the sizes of fishes. While

examining the gonads in connection with the present experiments I found some male fishes which gonads showed light milky white but much lighter than the usual male gonads. Microscopical examination revealed that the male gonad contained also a number of ova.

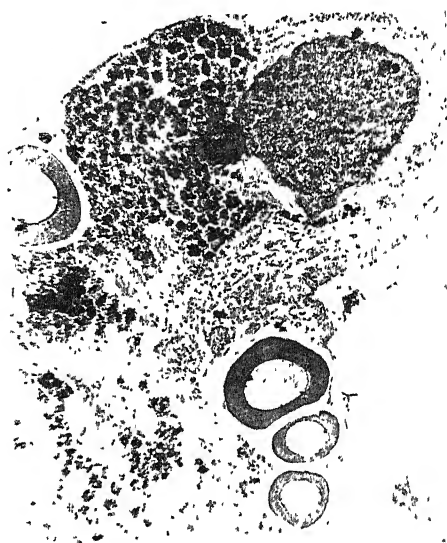


Fig. 3 Section of the ova-containing testis ($\times 100$)

In the picture (Fig. 3) the groups of spermatozoa will be noticed in the central part of the gonad and ova containing much dentoplasm. Both the fish measured 10 cm. in body length.

DISCUSSION

Since the present experiments were carried during April and December 1933, the temperature of water naturally showed marked difference. However all the fishes in each jar were kept under the same condition thus the alteration of temperature however large can be neglected as far as its effects on males and females are concerned.

Contrary to the expectation, the males of young *Carassius* showed definitely greater survival or resisting power than that shown by females against harmful environmental factors as presently tested.

It follows from the above, that we can not explain the preponderance of the females over the males by differential death rate, so far as the results of the present experiment are concerned.

Whether the presence of the ova-containing testis in *Carassius auratus*, showed the process of sex inversion as observed in *Xiphophorus*, *Triton*, *Rana*, and *Bufo* can not be said definitely, however, at least this fact suggests strongly a worth-wholeness of further research along this line in solution of extraordinary sex-ratio such as seen with the silver carp.

SUMMARY

- 1) The sex-ratio of *Carassius auratus* diverges considerably from 1 ♂ : 1 ♀ ratio.
- 2) Its sex-ratio changes regularly with the size of *Carassius auratus*.
- 3) Survival powers of males are greater against harmful environmental factors than females in young *Carassius*.
- 4) There is practically no difference in survival powers against harmful environmental factors of males and females in adult *Carassius*.
- 5) The "ova-containing testes" were found in two young *Carassius auratus*.

LITERATURE

- BADF, E. 1923. Süßwasser-Aquarium Berlin.
- CHAMPY, M. CH. 1921. Changement expérimental du sexe chez le *Triton alpestris* LAUR. C. R. de l'Acad. d. Sci., Tome 172.
- CREW, F. A. E. 1921. Sex-Reversal in Frogs and Toads. J. Genetics, Vol. XI.
- DÜRKEN, B. 1928. Lehrbuch der Experimentalzoologie. Zweite Aufl. Berlin
- DÜSING, K. 1883. Die Faktoren, welche die Sexualität entscheiden. Jenaische Zeitschr. f. Naturwiss., 16 N. F. 9.
- DÜSING, K. 1884. Die Regulierung des Geschlechtsverhältnisses bei der Vermehrung der Menschen, Tiere und Pflanzen. Jenaische Zeitschr. f. Naturwiss., 17 N. F. 10.
- ESSENBERG, J. M. 1923. Sex-differentiation in the viviparous Teleost *Xiphophorus helleri* HECKEL. Biol. Bull., Vol. 45.
- ESSENBERG, J. M. 1926. Complete sex-reversal in the viviparous Teleost *Xiphophorus helleri*. Biol. Bull., Vol. 51.
- GEISER, S. W. 1924. Sex-Ratio and Spermatogenesis in the Top-Minnow, *Gambusia holbrooki* Grd. Biol. Bull., Vol. 47.
- HARMS, W. 1921. Verwandlung des BIDDERSchen Organs in ein Ovarium beim Männchen von *Bufo vulgaris* LAUR. Zool. Anzeig., Bd LIII.
- JORDAN, D. S. 1925. Fishes. New York.
- KING, H. D. 1909-1910. Temperature as a Factor in the Determination of Sex in Amphibians. Biol. Bull., Vol. 18.
- KING, H. D. 1911. Studies on Sex Determination IV. Biol. Bull., Vol. 20.
- KING, H. D. 1912. Studies on Sex-determination in Amphibians. J. Exp. Zool., Vol. 12.
- KINOSHITA, T. 1933. A New Case of Hermaphroditism in *Carassius auratus* (L.). Jour. of Science of the Hiroshima Univ. Series B, Div. 1, Zoology, Vol. 2.

- KYLE, H. M. 1926. The Biology of Fishes. London
- MORGAN, T. H. 1928. The Theory of the Gene. Yale Univ Press
- NERESHEIMER, E. E. 1923. Die Fische. Leipzig
- PATTERSON, J. T. 1913. Polyembryonic Development in *Tatusia novemcincta*. J. Morph., Vol. 24. No. 4.
- SASAKI, K. 1926. On the Sex Ratio in *Carassius auratus*. Sci. Rep. Tôhoku Imp Univ. Biol., Vol. 1.

NOTES ON THE RELATION BETWEEN THE MOULTING, THE SEXUAL MATURATION AND THE LIGHT PERIOD IN *ZOSTEROPS PALPEBROSA JAPONICA*¹⁾

By

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(Received October 22, 1934)

In my previous paper²⁾, it has been stated that the sexual maturity of *Zosterops palpebrosa japonica* can be repeated at least three times a year by the prolongation of the daily period by using the light of an electric lamp. Actually, the third "Yogai" was begun on December 1, 1932, before completion of the moulting, in order to test how the "Yogai" would affect the moulting of the birds.

In Japan, among owners of pet birds, it is commonly known that the moulting appears inevitably after the stoppage of the "Yogai", and occurs normally and regularly, especially in a short period when the bird has been kept in a dim place even in the daytime, but that if a bird, which was ready to begin moulting, were put in a too bright place in the daytime, or in a corner of a lighted room in the evening (owing to carelessness or ignorance that the bird was being exposed to an imperfect "Yogai"), the moulting is prolonged and finishes very indistinctly, even after the stoppage of the "Yogai". Owners of pet birds used therefore to keep the birds in a place as dark as possible every evening after the "Yogai" had stopped, for the purpose of avoiding the prolonged and indistinct moulting, because they always hope to have graceful and well-shaped birds.

In the case of the third "Yogai" mentioned above, as expected, the number of falling feathers decreased after a few days from the beginning of the "Yogai", and a few birds apparently ceased moulting. But I mentioned nothing about this phenomenon in my previous paper, because I wished to re-examine the stoppage of the moulting and to report the result of more exact observation.

¹⁾The writer acknowledges his obligation to Prof. E. NOMURA for the publication of this paper

²⁾MIYAZAKI, H. 1934. On the Relation of the Daily Period to the Sexual Maturation and to the Moulting of *Zosterops palpebrosa japonica*. Sci. Repts. Tôhoku Imp. Univ., Biology, Vol. IX, Nos. 2 & 3, Pp. 184-203

The re-examination was carried out in my own laboratory in the city Kawagoe, Saitama-Ken, my native place. All the birds used for this part of the experiment were the same as those which were used in the preceding experiment mentioned in the third section of my previous paper, and were those which were exposed to the "Yogai" beginning on December 1, 1933, and which were transferred there from Sendai on April 18, 1934. In this case of this group of the birds, the "Yogai" was discontinued on June 1. As the result, the moulting began about ten days later.

It may be stated here as an interesting incident that five pairs of birds were allowed to escape from the bird cages on June 20. Unexpectedly, on the very day of the escape, one pair of them began to make their nest in a tree in the neighbourhood of my laboratory, with their plumage still in the process of moulting. About a week later four eggs were laid in this nest successively one every day. However, too frequent peeping into the nest, on my part owing to my desire to observe them thoroughly, caused a movement of this pair elsewhere out of sight. I could not determine decisively, therefore, whether the eggs were fertilized or unfertilized, even though it was most probable, from the direct observation of the blastodiscs, that the eggs were not fertilized. Any how, from this fact, it has been accurately ascertained that the gonad of this pair, and especially the ovary, was no longer in the stage of decreasing activity.

The "Yogai" was begun again for the remaining birds on June 25. The number of the falling feathers diminished considerably at the beginning of July. A few birds apparently stopped moulting, but the majority of them are continuing very slowly a prolonged and indistinct moulting even until to-day, September 19. By this date, the health of the birds has been remarkably impaired, the colour of their plumage having lost brightness. But the testes are still capable of sperm formation, even though their size seems to me a little smaller than that of the full mature specimens.

I am now in doubt whether the degree of sexual maturation of the gonad began at once to diminish in the transitional period from the second "Yogai" to the third, as stated in the first section of my previous paper. If the diminution to this degree were not the case, the sexual maturation, which appeared in the second and third "Yogai", must be perfectly continuous, and also in the present experiment, the sexual maturation which may finish at the beginning of June may be continuous with that in July. Yet, from the point of view that the degree of sexual maturation

is lower after the stoppage of the "Yogai" (compare Fig. 5 with Figs. 17, 19 and 21 in my previous paper), it may be stated that the full-sexual maturation can be regained artificially at least three times a year, and this fact especially becomes most reliable when we consider the one case in July of the present experiment.

According to a statement made by an owner of pet birds, a bird, which finished its moulting in June after a "Yogai" had ceased, underwent the natural moulting at the end of September, which is the regular period. I can say nothing about this phenomenon, as I have no data in connexion with it.

NOTE ON THE CHANGES OCCURRING IN THE BODY OF THE MALE NEWT (*TRITURUS PYRRHOGASTER*) DURING THE BREEDING SEASON

By

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(With Plate XI and 10 text-figures)

(Received November 5, 1934)

In the present paper I shall deal with some of the secondary sexual characters of the male of *Triturus pyrrhogaster* (BOIE), a kind of newt very common in Japan.

In 1930 UEKI published an article entitled "On the Sexual Differences in the Newt, *Diemyctylus pyrrhogaster* (BOIE)" and in this paper he alluded to the prominent morphological differences according to sex. Among these differences which he pointed out, we notice the following:

"In the males, the patroid glands and lateral glandular ridges develop especially during the sexually active period, and the vent not only swells but also many hair-like processes grow temporarily on its inside. In the sexual season the male is beautifully multicolored, contrary to the simple black or brown-black colour of the female. At this period the skin of the male becomes soft and velvety and the sex can be distinguished by mere touch with the fingers" (UEKI, '30, p. 147-148).

His observations were chiefly done macroscopically and thus much seems to remain to be studied more precisely.

The main purpose of the present paper is to observe precisely the following facts which will be seen in the male newt during the breeding season, viz. 1) the change of colour of the body surface, 2) a remarkable growth of the cheek processes which contain the hedonic gland, 3) the hair-like processes growing inside of the vent, and 4) the increase in length of the narrowed terminal portion of the tail.

Here I wish to express my sincere thanks to Prof. S. HÔZAWA, at whose suggestion this research was undertaken and under whose helpful leading it was carried out. I wish also to thank Assistant Professors S. NOMURA and I. MOTOMURA for many valuable hints and kind courtesies extended to me during the study.

MATERIAL AND METHOD

The newt used in the present study for material were collected at several localities distributed in the suburb of Sendai.

To anaesthetize and kill the animal 0.3 per cent solution of chlorotone was used and thus good results were obtained. In fixing material for histological purpose I have tried various fluids commonly used. Of the skin of the newt it is well-known that to obtain good section is in most cases rather difficult. But this difficulty was removed by using Bouin's fluid.

The sections were prepared by paraffin method and were cut 8-15 μ thick. In staining the sections various stains were tried, each being used singly or in combination with one or more of the others. Above all, the combination of Heidenhain's iron-haematoxylin with orange G, and the same of Delafield's haematoxylin with eosin were successfully used in most cases. To differentiate the elastic tissue from the others, both of the fuchsin-indigo-picric acid staining and Weigert's resorcin-fuchsin combined with Van Gieson's mixture were employed. For the connective tissue, Mallory's stain was tried, and for the collagenous tissue Heidenhain's modification of the same was used. Thionin was regarded as a good reagent to stain mucous secretion. To trace the nerves distributed inside the hair-like appendages in cloaca I have tried Schultze's method modified by MOTOMURA and thus I was able to obtain a good result. For the same purpose I tried also the method of vital staining with methylene blue, but it was not successful.

(1) The Change of Colour of the Body Surface.

Among the secondary sexual characters of the male newt which will appear during the breeding season, the change of coloration of the body surface is most remarkable and strongly attracts our attention (Pl. XI, figs. 4, 5, 6).

In the ordinary season the colour of the male newt is blood red on the ventral surface of the body, this colour extending from the tip of the mouth to the tail end, and is black in the remaining portion. The blue colour is almost not observable in this case, though it is rarely seen only in a small area located near the tail end. When the winter is over and the spring approaches, the blue colour begins to appear on the body surface of the male newt first in the tail region and then on the ventral surface of the posterior trunk. The area coloured blue spreads gradually

wider towards the head and thus in the middle of the breeding season it covers nearly all of the body-surface, leaving only the dorsal surface of the trunk. When the breeding season is finished the blue colour above mentioned begins to disappear. In this case the tail which was coloured first loses its colour very late while the head which was coloured later will fade first.

(2) The Cheek Processes

During the breeding season a remarkable growth of the cheek processes take place in the male newt (Pl. XI, figs. 4, 5). Each of these processes projects horizontally and outwardly from the posterior corner of the head and they contain numerous glands (Text-fig. 1, *gl. grn*). These processes measure only 2.2 mm. in breadth in ordinary season, while in the breeding season it becomes nearly twice as big. The minute structures of these cheek processes may be mentioned below.

(a) *The epidermis.* The epidermis (Text-figs. 1 and 2, *ep*) consists of from two to about seven cell-layers, the cell-layer which is in contact with the dermis is composed of columnar cells forming the germinative layer. Several cell-layers which come to the next namely the germinative layer consist of polygonal cells and form the stratum mucosum.

One or two cell-layers lying outside of the stratum mucosum are composed of flattened cells. The outermost cell-layer of the epidermis is composed of cornified cells and this layer will be stripped off during the moulting.

(b) *The dermis.* The dermis (Text-fig. 2) may be divided into three layers, viz. the outer compact layer (*drm'*), the intermediate spongy layer (*drm''*) and the inner compact layer (*drm'''*).

The outer layer is very compact in structure and the connective-tissue fibres composing this layer run in most cases parallel to the surface of the body, but sometimes some of these fibres run perpendicularly to the body surface distributing among the glands.

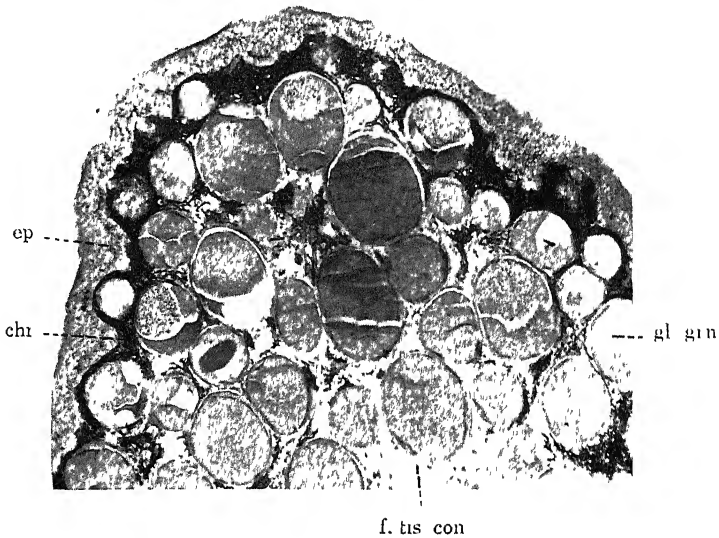
The intermediate sponge layer is chiefly composed of loosely woven bundles of connective-tissue fibres (*f. tis. con.*) The fibre-bundles run rather irregularly taking various directions. This layer also contains many lymph spaces distributed irregularly.

The inner compact layer is not very compact as in the case of the outer layer.

The most of the connective-tissue fibre bundles which constitute the bulk of this layer run parallel to the body surface while some of these

bundles run rather irregularly without regard to the orientation

Of these three layers above mentioned the intermediate layer is superior in thickness to any of the remaining two, and becomes specially thick during the breeding season. The fact that the cheek processes of the male newt become very voluminous during the breeding season is due to the increase of the thickness of the connective tissue layer (*tis. con.*) of



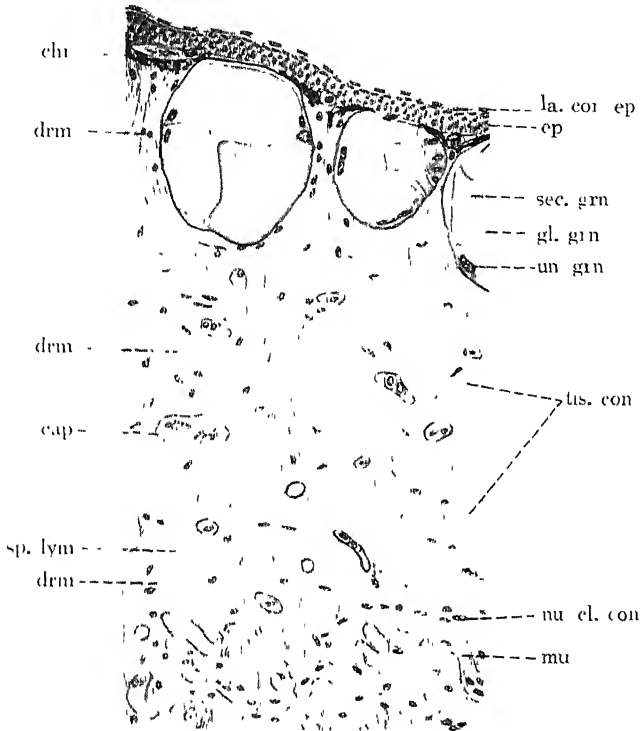
Text-fig. 1 The cheek process of the male newt in the breeding season. A section cut through underneath the epidermis and parallel to the outer surface of the process ($\times 70$). *ep* - epidermis, *chr* - chromatophore, *gl. gin.* granular gland, *f. tis. con.* - connective tissue fibre.

the intermediate layer (*drm''*). The other two layers of the dermis do not increase their thickness even in the breeding season and thus they remain almost constant through all seasons

(c) *The blood supply.* A large vessel enters into the inner layer of the dermis (Text-fig. 2, *drm'''*) taking the course first outwards along the mid axis of the process, and then it turns backwards parallel to the body axis giving off many branches on its way. Many capillaries (Text-fig. 2, *cap.*) may be found between the epidermis and the outer compact layer of the dermis, but the outer layer of the dermis is supplied with only a small number of these capillaries.

(d) *The pigment.* A thick layer of pigments (Text-figs. 1, 2, *chr.*) is deposited in the outer compact layer of the dermis, though they do not

form a complete continuous sheet. In addition to the above, a small number of pigment cells are found in both of the stratum mucosum and the stratum germinativum. The same kind of cells are scattered among the outer and intermediate layers of the dermis.



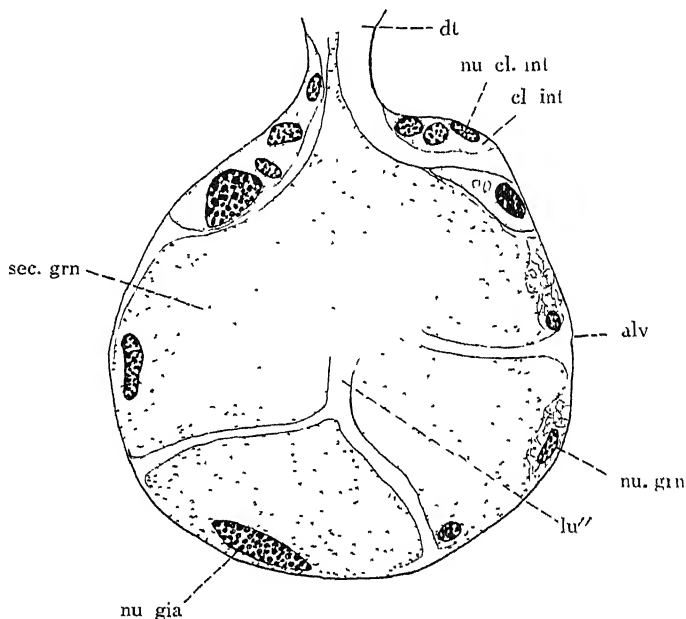
Text-fig 2. Cross-section of the cheek process of the male newt in the breeding season ($\times 80$) *cap.*—blood capillary; *chr.* chromatophore; *drm'*—outer compact layer of dermis, *drm''*—intermediate spongy layer of dermis; *drm'''*—inner compact layer of dermis; *ep.* epidermis; *gl. grn.*—granular gland; *la. cor. ep.*—cornified epidermal layer; *mu.*—muscle, *nu cl. con.*—nucleus of connective-tissue fibre; *nu grn.*—nucleus of granular cell, *sp. lym.*—lymph space; *sec. grn.* granular secretion; *tis. con*—connective tissue

(e) *The nerve.* Concerning the nerves which supply these structures we may say that a large nerve fibre bundle comes into the middle layer of the dermis and then it divides into many small branches which distribute in all parts of the process.

(f) *The elastic tissue.* To stain the elastic tissue I have tried Weigert's resorcin-fuchsin combined with Van Gieson's mixture. Thus the elastic fibre, connective-tissue and muscles were stained black, red and yellow respectively.

The elastic fibres which may be found everywhere in the dermis run in most cases parallel to the connective-tissue bundles, but they rarely intercross with others forming irregular networks. Each of these elastic fibres is divided into many branches and these branches thus formed may be found very numerous in the inner compact layer of dermis. The elastic fibres occur very abundantly in the intermediate spongy layer and are superior in quantity to the connective-tissue fibres.

(g) *The gland.* A large number of glands exist in the dermis (Text-figs 1 and 2, *gl. grn*). They are of the simple alveolar type (Text-fig. 3) and are distributed between the outer and the intermediate layer of



Text-fig. 3 Median longitudinal section of a well-developed granular gland of the male newt. ($\times 300$). *alv.* - alveolus, *cl. int* - intercalary cell; *dt* - duct; *lu''* - lumen of granular gland; *nu. cl. int.* - nucleus of intercalary cell, *nu. grn* - giant nucleus, *sec. grn* - granular secretion.

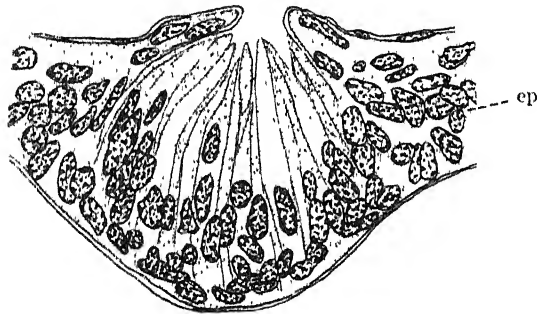
the dermis. These glands have been divided by several writers into three groups after their characteristics viz. 1) the large granular or poisonous gland, 2) the small mucous gland and 3) the gland of mixed type, that is partly granular and partly mucous. According to this designation, the gland of this case should belong to the first group for the following

reasons: 1) there exist smooth muscles surrounding the gland, while there are no such muscles in the case of the gland of mucous type; 2) the secretion mass from this gland takes the ordinary plasm stains very readily, showing always some decided colour. Practically, the content of this gland was stained red or dark purple with Mallory's mixture and yellow with Van Gieson's stain.

But it is never affected by the basic stains as safranin, fuchsin etc., as in the case of the contents of the gland of the mucous type. On the contrary, the gland found in each hair-like appendage is small and is of mucous type and thus its secretion which I will describe later, was stained purple by thionin and red by fuchsin.

Each of the granular glands (*gl. grn.*) consists of three parts: the duct (*dt.*), intercalary region (*int.*) and alveolous (*alv.*). The duct is cylindrical and measures about 33μ in length and 13μ in breadth. It opens to the exterior passing through first the outer layer of the dermis and then the epidermis. The intercalary region is made up of from 8 to 15 cells arranged in ring-like manner around the base of the duct. The boundaries of these cells are not very distinct, and their nuclei are elongated oval in shape with their longer and shorter diameter of 13μ and 3μ .

The main body (*alv.*) of the gland is spherical or somewhat ovoid in form, the longer and shorter diameter attaining respectively up to 280μ



Text-fig. 4. Longitudinal section of a young granular gland found in the cheek process of the male newt in the breeding season ($\times 300$)
ep. epidermis.

and 210μ . The entire outer surface of the gland body is covered by closely set connective-tissue fibres which are continuous with those of the outer and intermediate layers of the dermis. In addition to these con-

nective-tissue fibres there may be found fine elastic fibres too. Inside of the said layer of connective-tissue fibres there lies a layer of muscle fibres and inside of this comes the epithelium of the gland. The gland alveoli (*alv.*) are completely filled up with dense granular secretion (*sec. grn.*). At the base of each gland cell a number of nuclei may be seen. In sections these nuclei look variously shaped, viz. spherical, oval, elongated oval, etc. Among these nuclei there exists a very large one which is called "giant nucleus" (*nu. gia.*). It measures about 70μ in its longer diameter, and contains many large chromatins. The above descriptions may be applied chiefly to the fully-grown glands. Among the fully-grown glands we also find those which are not yet fully grown. The latter look somewhat different in appearance from the former as shown in text-fig. 4.

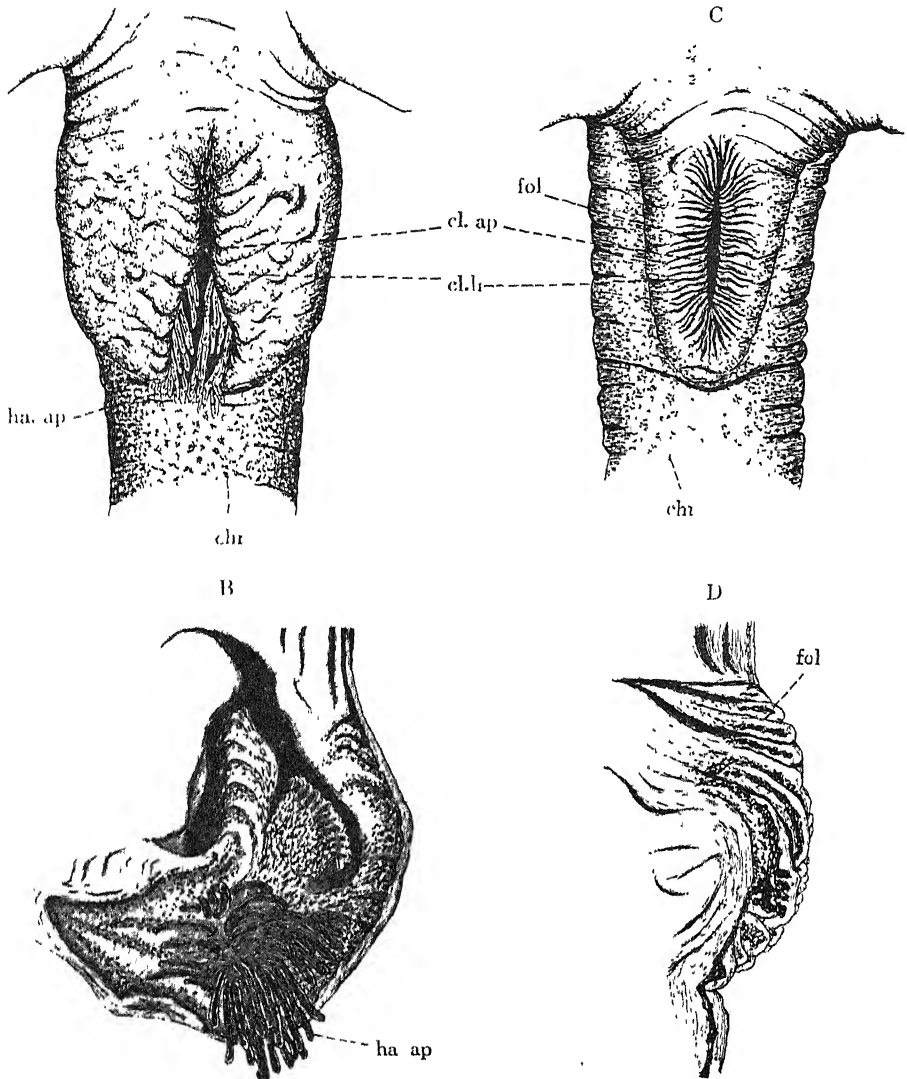
The gland above alluded to is holocrine. In some case the cell-wall of each gland cell is quite distinct being observed in the basal portion of the cell and thus the cell boundary may be rather distinct, while in the apical portion of the cell, it is mostly obliterated and thus the cell-boundary is very obscure. In other cases, the cell-wall is entire and thus it surrounds all the surface of the gland cell

(3) The Hair-like Appendages found in the Cloaca.

In 1912 KÜKENTHAL published an article concerning the hair-like appendages of an African frog, *Astylosternus robustus* (BLGR.). They are found only in the male frog during the breeding season and are distributed on the flanks of the body and on the thighs of the legs. Each of these appendages is finger-like in shape and attains a length up to 20 mm. Externally it is surrounded by a dark-coloured membrane continuous to the skin covering the body and internally there is an axis composed of dense connective tissue. Of these hair-like appendages KÜKENTHAL concluded that they are to be regarded as a secondary sexual character of the male of this kind of frog.

In the case of *Triturus pyrrhogaster*, we notice also hair-like appendages resembling in some respects those of the African Hairy frog above mentioned. In this case again they are present only in the male, but are found in the cloacal cavity, not on the outer surface of the body-skin. Differing from the case of the Hairy frog they are seen through all seasons of the year, but they become very distinct in the breeding season attaining their maximum length and thus their distal extremities projecting

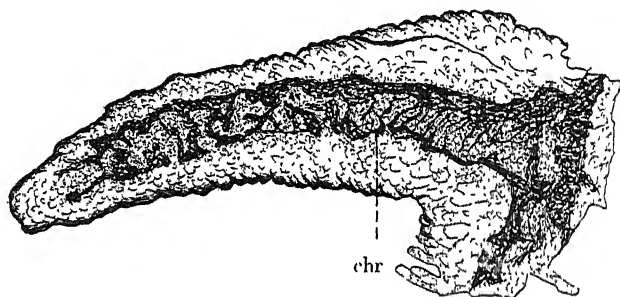
outside of the cloacal aperture. In other seasons of the year they are very short, and are entirely hidden in the interior of the cloacal cavity.



Text-fig. 5. *Triturus pyrrhogaster*. A—Cloacal region of the male in breeding season, showing the hair-like appendages (*ha. ap.*) (ca. $\times 6$). B—The same cut sagittally along the median line to show the hair-like appendages (*ha. ap.*) growing inside of the cloacal cavity (ca. $\times 7$). C—Cloacal region of the female in the breeding season (ca. $\times 6$). D—The same cut sagittally along the median line (ca. $\times 7$).

When fully grown they are from 0.5 mm. to 2 mm. long by 0.2 mm. thick in the middle. Thus it seems to be very reasonable to recognize this feature as one of the secondary sexual characters of the male of the Japanese newt.

In external appearance the hair-like appendages of the Japanese newt are essentially similar to those of the African Hairy frog, but internally there may be noticed some marked differences. Of the hairy appendages of the African frog KÜKENTHAL says that the stratum corneum is quite distinct though it is not thick, and the epidermal outer layer is made up of many longitudinal ridges of epidermal cells, between which are found deep longitudinal grooves, which are filled with the cutis tissue. He describes elsewhere: "There is a quite conspicuous blood vessel running along the long axis of the cutis papilla and other smaller blood vessels are found in the surrounding substance of the cutis. The whole papilla is built up of a dense connective tissue, Chromatophores are numerous, being especially abundant at the base of the appendage . . . that

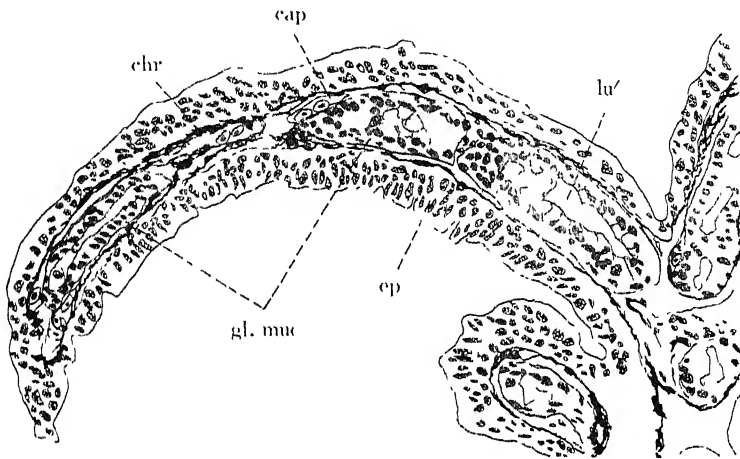


Text-fig. 6. One of the hair-like appendages growing inside of the cloaca of the male newt in the breeding season. External view (ca. $\times 70$) chr. - chromatophores.

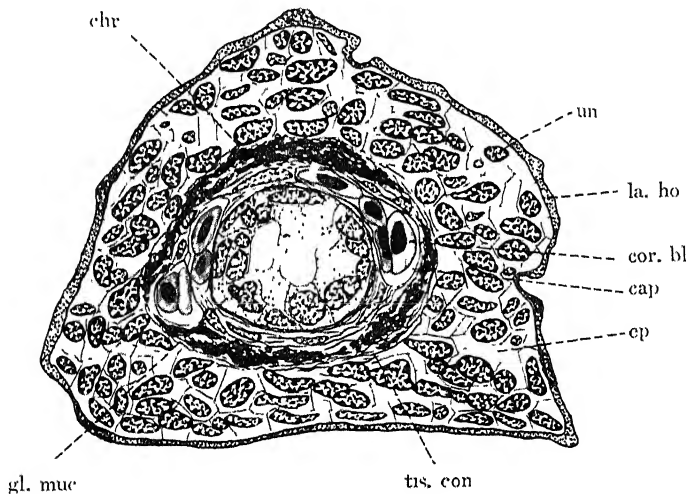
there are both nerves and nerve-terminations in these appendages, and that therefore they do serve as sensory organs". "They (Tactile cells) are situated in the grooves between the epidermal ridges. Each of them is provided with an axis-cylinder, which ran quite close to the surface of the epidermis, but in the cutis tissue beneath it, and were united proximally into a common nerve fibre" (pp. 374-375).

Of the hairy-appendages of the newt it may be mentioned as follows. Each of these appendages (Text-fig. 5, A and B and text-figs. 6, 7, 8

and 9) is surrounded by a layer of stratum corneum. The epidermis is very thick, its thickness being about the half of the diameter in cross-section of the appendage and its component cells are arranged irregularly



Text-fig. 7 Median longitudinal section of a hair-like appendage, showing the tubular mucous gland ($\times 100$). *cap* - blood capillary, *chr* - chromatophore, *ep* - epidermis, *gl. muc* - mucous gland, *lu* - lumen of mucous gland

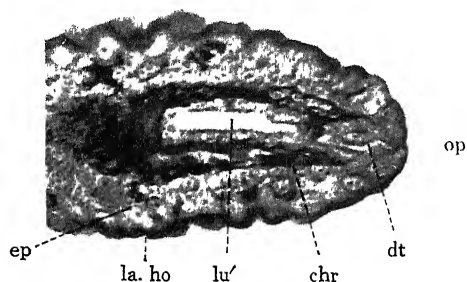


Text-fig. 8. Cross-section of a hair-like appendage ($\times 325$). *cap* - blood capillary; *chr* - chromatophore, *cor. bl* - blood corpuscle, *ep* - epidermis; *gl. muc* - mucous gland; *la. ho* - horny layer; *un* - nucleus; *tis. con* - connective tissue.

in from one to five layers. The demarcation of these cells are rather distinct. Nuclei are somewhat ovoid measuring about 18μ in length by 8μ thick.

The chromatophores (*chr.*) are very numerous, being found beneath the epidermis. Thus the pigment they contain makes it very difficult to observe the minute internal structure of the appendage, especially in order to trace the nerve fibres.

A large tubular mucous gland (Text-figs. 7 and 8, *gl. mc.*) runs longitudinally along the axis of the appendage, its proximal end being communicated with the pelvic gland. Thus this gland is but the prolongation of a branch rising from the pelvic gland. The gland consists of merocrine



Text-fig 9 Photograph of a section cut slightly obliquely through the terminal portion of a hair-like appendage, showing the internal lumen of the duct of the mucous gland and the external opening of the duct ($\times 170$). *chr.*—chromatophore, *dt.*—duct of mucous gland, *ep.*—epidermis; *la ho*—horny layer; *lu'*—lumen of mucous gland, *op.*—opening of the duct of mucous gland.

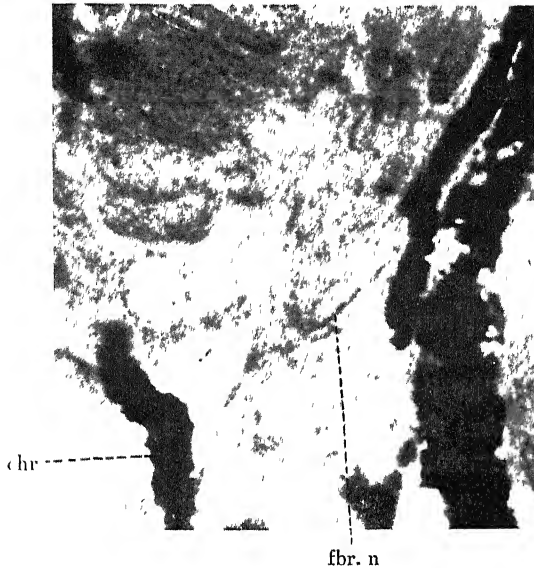
cells arranged in single or two layers enclosing the lumen which opens to the exterior by means of an opening provided at the tip of the appendage. The limits between these gland cells are observable and their irregularly shaped nuclei are also clearly visible. The mitotic nuclear division was rarely observed in the case of these gland cells. The secretion of this gland appears to be fibrous when observed in the fixed material. The content of this gland may be stained blue

by Mallory's connective-tissue staining, red by Weigert's staining combined with that of Van Gieson, and purple with thionin. This secretion may not be stained with Heidenhain's iron-haematoxylin, while it becomes blue stained by Delafield's haematoxylin. Two or three blood capillaries run longitudinally inserted between the pigment layer and the outer wall of the mucous gland. Their extremities are traceable to a point located a little distant from the tip of the appendage.

The connective-tissue layer is very thin, lying between the pigment layer and the outer wall of the mucous gland.

To trace the nerves distributing the appendage (Text-fig. 10, *fbr. n.*), Cajal's and Bielschowsky's methods were tried, but good results were not

obtained, but in applying MOTOMURA's method¹⁾ I was successful in finding some nerve fibres which enter the appendage from its base and run along



Text-fig. 10 A photograph showing the nerve fibres distributing in the hair-like appendage ($\times 900$) *chr* - chromatophore, *fbr. n* - nerve fibre.

the inner side of the pigment layer. These nerve fibres seem to have arisen from the fifteenth spinal nerve.

Concerning the function of these hairy appendages I can not conclude definitely, but judging from the facts I noticed that a great number of

¹⁾ SCHULTZE's method modified by MOTOMURA to obtain paraffin section.

The procedure is as follows

1. 20% formol 2 days.
2. 1/5 N. NaOH 1 days.
3. Wash in water 8 hrs
4. 1% AgNO₃ 3-7 days at 35-40°C.
5. Dehydration.
6. Paraffin section
7. 80% Alcohol for a moment
8. Reduction in hydrochinon 4 grm. formol 10 cc. dist. water 100 cc. 24 hrs.
9. (sat. sol. of hypo).
10. Alc of increasing concentrations up to abs Alc.
11. Xylol.
12. Mounting

spermatozoa adhere to the outer surface of the appendages and the same were seen involved in the mucous secretion of the appendage, I may safely say that this structure will be profitably used to retain the spermatozoa until the chance of fertilization comes.

(4) The Increase in Length of the Narrowed Terminal Portion of the Tail.

The fact that the narrowed terminal portion of the tail of the male newt will be increased in length during the breeding season, was formally observed by K. TAGO in the case of the same kind of newt. He says that the length increased is from 8 mm. to 10 mm. But in the case of my own observation it was from only 1.5 mm. to 6 mm.

In the act of courtship, the male newt first bends his tail toward the female and then shakes it fairly rapidly, thus the narrowed terminal portion of the tail touches the neck of the female and gives some mechanical stimulation to the female.

SUMMARY

In the following, the most remarkable changes which occur in the body of the male *Triturus pyrrhogaster* will be summarized. These changes occur only in the male and moreover only during the breeding season and thus they may be looked upon as the secondary sexual characteristics of this kind of newt.

1. During the breeding season a blue colour will appear nearly all over the surface of the body excepting for the dorsal surface of the trunk. The colour begins to appear when the hibernation is finished and it becomes very distinct and beautiful at the climax of the breeding season. When the breeding season is ended, this blue colour will gradually disappear. Thus it seems to be quite reasonable to mention that this beautiful colour is of sexual meaning, being utilized by the male newt as a mean of attracting the female.

2. During the breeding season, the hair-like appendages which are found inside the vent will grow markedly and thus their length will be nearly doubled. They are thought to be the projections of the skin and are covered by a very thick epithelium continuous to that of the skin. Each of these processes contains inside a large tubular mucous gland, chromatophores arranged in layers, a small quantity of connective-tissue, a small number of blood capillaries, etc.

A number of fine nerve fibres were observed distributed inside of each process. The function of these structures may be assumed to be sexual. The mucous secretion which they produce will protect the spermatozoa involved in it until the chance of fertilization will visit them and moreover will activate their motion in performing the fertilization

3. The cheek processes will grow enormously and will become very voluminous during the breeding season. It is due to a very rapid increase of the connective-tissue contained inside of the process. In the interior of this process there are found a great number of granular gland.

The cheek process may also be assumed to be sexual. It seems to give some mechanical stimulation to the female by touching it to the body of the latter.

4. During the breeding season the terminal portion of the tail which is much narrower than the other portion will be distinctly prolonged. This prolongation of the tail end seems also to be of sexual meaning, being utilized by the male in giving some mechanical stimulation to the female.

LITERATURE CITED

- BOULENGER, G. A. 1901 Further notes on the African Batrachians *Trichobatrachus* and *Gampsosteonyx*. Proc Zool. Soc. London, Vol. 2, pp 709-710
- BAWSON, A. B. 1920. The integument of *Necturus maculosus*. Jour. Morph., Vol 34, pp. 487-577.
- GÔDA, T. 1930. Soshikigaku. Iwanami's Seibutsugaku.
- KÜKENTHAL, W 1908-1913. On the hair-like appendages in the frog *Astylosternus robustus* (BLGR.). Bull. Mus. Comp. Zool., Vol. 53, pp. 371-376.
- NICOGLU, P. 1893. Über die Hautdrüsen der Amphibien. Zeit. f. wiss. Zool., Bd. 56, pp. 409-485.
- NOBLE, G. K. 1931. The biology of the Amphibia. 1st ed.
- TAGO, K. 1930. The Salamanders of Japan.
- UEKI, T. 1930. On the sexual differences in the newt, *Diemyctylus pyrrhogaster* (BOIE). Sei Rep. Tôhoku Imp. Univ. Biol., Vol. 5, No. 1, pp 133-151.

EXPLANATION OF PLATE XI.

Triturus pyrrhogaster in the breeding season, showing the secondary sexual characters.

Fig. 1. The female; dorsal view (natural size)

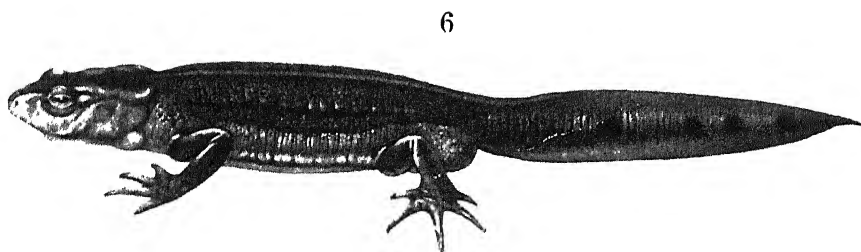
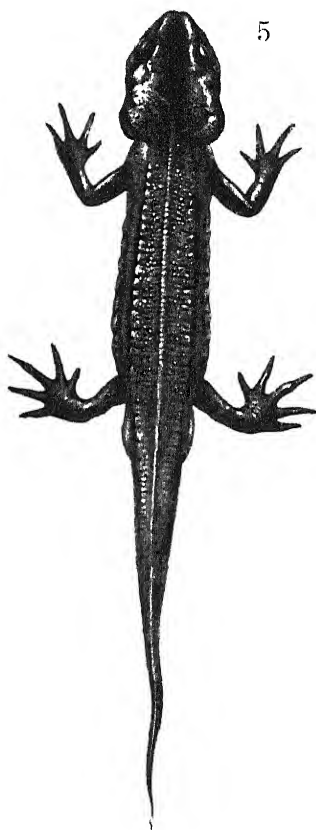
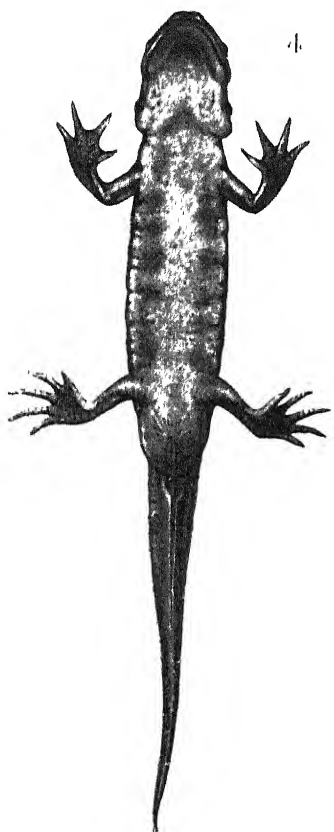
Fig. 2. The same; ventral view (natural size).

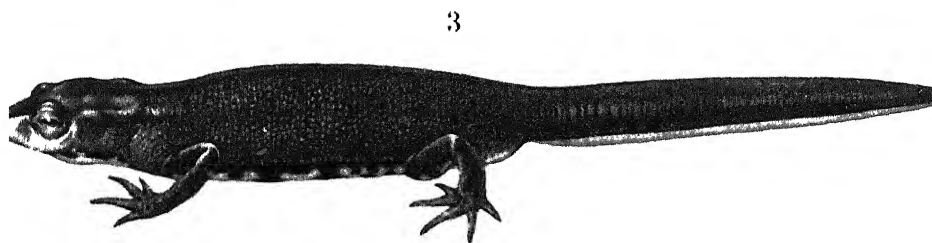
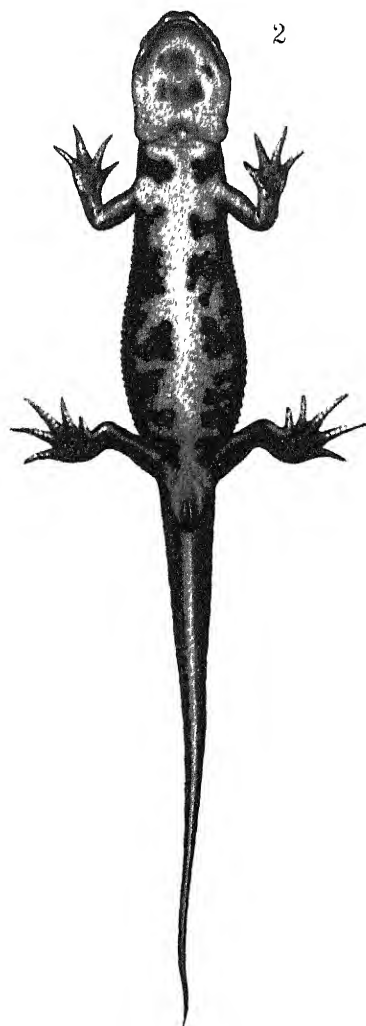
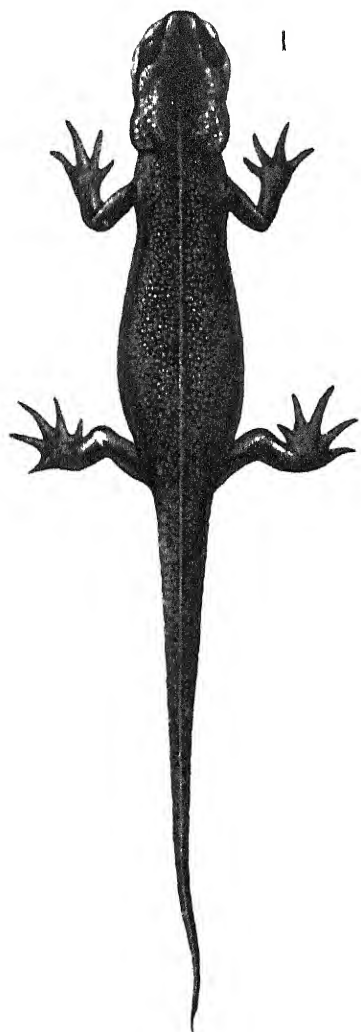
Fig. 3. The same; lateral view (natural size)

Fig. 4. The male, ventral view (natural size).

Fig 5. The same, dorsal view (natural size).

Fig 6. The same, lateral view (natural size).





ON THE STRUCTURE OF ANUS OF A HOLOTHURIAN, *CAUDINA CHILENSIS* (J. MÜLLER)

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(With six figures)

(Received November 10, 1934)

In 1927, KAWAMOTO contributed an article concerning the anatomy of *Caudina chilensis* (J. MÜLLER) and in that article he mentioned that the coelomic cavity of this animal opens to the exterior by means of five canals, each of which having the external openings placed at the inner base of each anal papilla. To these canals he has given the name of coelo-anal canals.

In my previous paper published in 1933, I dealt with the anatomy of the young forms of the same species and reported that the coelo-anal canals mentioned by KAWAMOTO were not found in that case. In his personal correspondence Dr. GEROULD has given me the following notice that my failure to find the coelo-anal canal is in line with the idea expressed to him by Prof. H. L. CLARK that it is a lesion, the artificial result of injury, when found in the adult.

In the present paper I should like to deal with the morphology of the anus of this kind of animal and to take the so-called coelo-anal canals into special consideration.

OBSERVATION AND DISCUSSION

I have observed the tail end of this kind of *Caudina* of the individuals of various stages preparing the sections cut crosswise and longitudinally.

Approaching the anus each of the radial water canals increases its calibre (fig. 2) and there it gives off one or two very small branches on each side (fig. 1). The main trunk of the radial water canal enters finally into the central (primary) anal papilla, while each of the small branches above mentioned enters into each of the lateral (secondary or tertiary) papillae (fig. 1). These side branches are in the form of very short and narrow canals and thus it is rather hard to observe them even under high magnification.

In the case of the fully grown anal papillae which ampullae were called as circumanal ampullae by DENDY (1898), the cavity of each ampulla

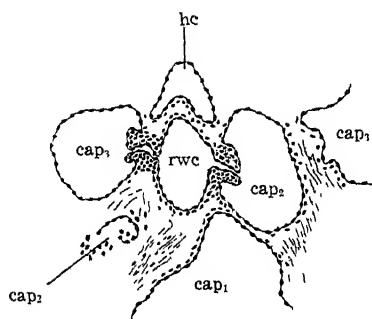


Fig. 1 *Caudina chilensis*. Cross section to show the radial water canal branching at the posterior end. Adult. $\times 150$. *cap*₁, circumanal ampulla of the primary anal papilla; *cap*₂, the same of the secondary one; *cap*₃, the same of the tertiary one, *hc*, hyponeural canal, *rwc*, radial water canal

anal papillae, three of them occurring in each radius (Table II). In addition to these anal papillae we notice five anal teeth, each of which is placed in each radius (fig. 3, *at*).

I have appended here two tables showing the number of anal papillae and of the so-called coelo-anal canals found in various stages of growth in the cases of *Caudina chilensis* and of *Molpadia roretzii*. I should like to mention that these individuals with damaged tail end are not included in these tables.

In the case of *Caudina chilensis* the longitudinal muscle bands of the body gradually diminish their thickness towards the tail end and when they arrive at the point where the radial water canal is swollen (fig. 5) the separation into two halves becomes rather obscure. Finally they join the longitudinal muscle fibres found in the inner wall of the circular anal cavity (fig. 2). The outer wall of the circular anal cavity where the longitudinal muscle bands do not exist is built up by circular muscle fibres distributed in the form of a band and surrounds the circular anal cavity (fig. 2, *cmb*).

The circular anal cavity (Text-fig. 2, *cac*) which surrounds the anus is

is continuous with that found in the interior of each papilla, but in the case of the papilla not fully grown the cavity of each ampulla remains only in the form of an oval sack. These papillae, not fully grown, correspond to the rudimentary ambulacra which were reported by GEROULD (1896) in the case of *Caudina arenata*. The number of the anal papillae is rather variable in the individuals of various growths (Table I), but it is certain that five papillae will appear at some stage during the life of this animal, each being placed in the position of each radius.

In the case of *Molpadia roretzii* (von MARENZELLER), we find fifteen

TABLE 1*
Caudina chilensis.

Body length in mm.	Number of anal papillae found in each radius					Number of so- called coelo-anal canals
	I	II	III	IV	V	
9	3	3	3	3	3	0
10	3	3	3	3	3	0
10	3	3	3	3	3	0
10	3	3	3	3	3	0
11	3	3	3	3	3	0
26	3	3	3	3	3	0
33	3	3	3	3	3	0
34	3	3	3	3	3	0, 1 d
35	3	3	3	3	3	0
39	4	4	3	3	3	0, 3 d
42	4	4	3	3	3	0
45	1	4	3	4	3	5
50	5	5	5	5	5	0
50	5	5	5	4	4	0
50	5	5	5	3	4	1, 1 d
53	5	3	{ 4 (1)	1	3	0
55	5	3	2	4	3	0, 5 d
55	5	5	5	5	5	0
55	3	3	3	3	3	0, 5 d
60	{ 1 (1)	{ 4 (1)	1	4	1	0, 2 d
65	4	1	3	3	3	5
68	3	3	3	3	{ 2 (1)	0, 3 d
75	5	5	4	4	4	1, 4 d
75	4	4	4	3	3	0, 5 d
80	3	3	3	3	3	1, 4 d
90	3	{ 2 (1)	{ 2 (1)	{ 2 (1)	{ 2 (1)	0
90	{ 2 (1)	{ 2 (2)	{ 2 (2)	{ 2 (2)	{ 2 (2)	0, 3 d
95	{ 4 (1)	{ 2 (1)	3	4	4	1, 2 d
100	5	5	5	5	5	5
105	3	3	3	3	3	0
105	{ 3 (2)	3	{ 3 (1)	{ 3 (2)	3	5
105	{ 2 (1)	{ 1 (2)	{ 1 (2)	{ 1 (2)	{ 1 (2)	0, 2 d
110	{ 2 (1)	{ 1 (2)	{ 1 (1)	{ 1 (1)	{ 1 (1)	5
140	3	3	3	3	3	0
115	5	5	5	5	5	5
180	5	5	5	5	5	0
195	{ 3 (1)	3	3	3	3	5
230	3	2	1	1	1	0
240	4	{ 2 (1)	{ 2 (1)	3	{ 2 (1)	0, 5 d
240	{ 4 (1)	5	(5)	(2)	(5)	0
270	3	3	3	{ 1 (2)	{ 1 (2)	0, 1 d

TABLE II.*
Molpadia roretzii

Body length in mm	Number of anal papillae found in each radius					Number of so-called coelo-anal canals
	I	II	III	IV	V	
50	3	3	3	3	3	0
115	3	3	3	3	3	0
115	3	1	2	3	2	0
120	{ 1 (2)	{ 1 (2)	{ 1 (2)	{ 1 (2)	{ 1 (2)	0
125	3	3	{ 1 (2)	{ 1 (1)	{ 2 (1)	0

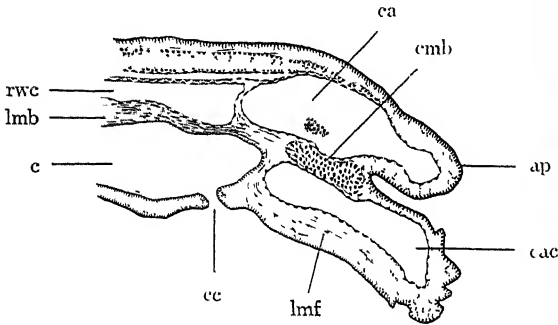


Fig. 2. *Caudina chilensis*. Longitudinal section of the tail end. Specimen about 50 mm long \times 100 μ , anal papilla; c, coelom; ca, circumanal ampulla, cac, circular anal cavity; cc, coelo-anal canal, cmb, circular muscle band; lmb, longitudinal muscle band; lmf, longitudinal muscle fibres; rwc, radial water canal

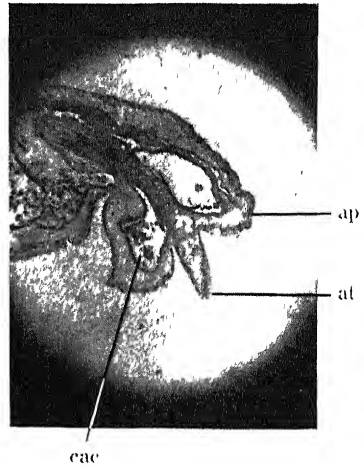


Fig. 3. *Molpadia roretzii*. Longitudinal section of the tail end. Adult. $>50 \mu$, anal papilla; at, anal tooth; cac, circular anal cavity.

separated and differentiated from the coelom and has not any communication with it. However, we often notice a number of blood corpuscles gathering in this cavity. The circular anal cavity seems to have some relation to the movements performed by the anus in opening and closing. In the case of *Caudina chilensis* the said cavity is more fully developed

* In these tables, the figures (1), (2), (5) denote the number of the rudimentary anal papillae and the figure 'd' denotes the so-called coelo-anal canal which is on the way of formation; for instance, '1, 4 d' denotes that there exists one so-called coelo-anal canal and 4 of those which are on the way of formation

in the young form of over 20 mm. body length than in the adult. The author has observed the same kind of cavity also in the cases of *Molpadia roretzi* (fig. 3, *cac*) and of some other species belonging to the genus *Caudina* (fig. 4, *cac*). In the case of *Caudina arenata* GERROULD so illustrated a cavity which seems to correspond to this circular anal cavity (GERROULD 1896, Pl. 1, Fig. 50).

Now I shall deal with the structures which were called by KAWAMOTO as coelo-anal canals. Each of these canals opens into the anus by means of an orifice situated at the inner base of each anal papilla in radial position and in other words in the cloacal wall located anterior to the circular anal cavity (fig. 2, *cc*). In the specimens of over 30 mm. body length, I have observed that the cloaca is surrounded by a wall of uniform thickness and there is not found

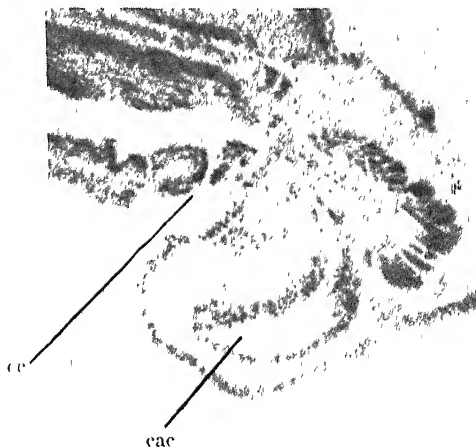


Fig. 4. *Caudina* sp. Longitudinal section of the tail end Adult. $\times 140$ *cac*, circular anal cavity, *cc*, coelo-anal canal

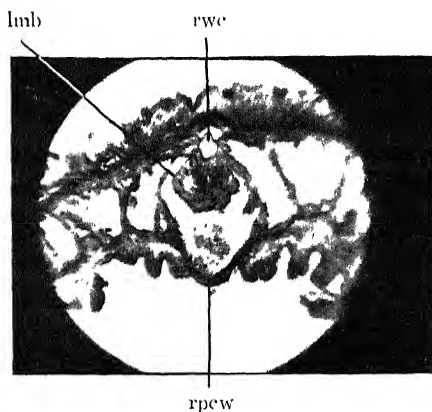


Fig 5 *Caudina chilensis* Cross section to show the so-called coelo-anal canal developing Adult $\times 60$ *lmb*, longitudinal muscle band; *rpcw*, radial portion of the cloacal wall; *rwc*, radial water canal

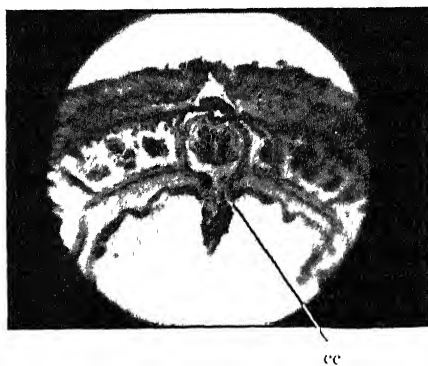


Fig. 6. *Caudina chilensis* Cross section to show the so-called coelo-anal canal. Specimen about 100 mm. long $\times 60$. *cc*, coelo-anal canal

any orifice through which the cloaca communicates with the coelom. But we often notice cases where a part of the cloacal wall placed in radial position is pushed towards the cloacal cavity in the form of a hollow process and thus the wall covering that process becomes very thin (fig. 5, *rpciv*) and that it is finally perforated leaving a canal in that place (fig. 6, *cc*). This canal thus formed may be a so-called coelo-anal canal and the number of these canals varies from one to five according to the case (Table I). The said canal is very narrow and is often filled up with blood corpuscles gathered there. The fact that in the young specimen of less than 30 mm. body length there do not exist so-called coelo-anal canals was already mentioned in my previous report.

We can easily imagine the pressure which will be produced by the body fluid contained in the coelom when the animal suddenly contracts under some condition. It can be easily imagined again that the pressure thus produced will influence the wall of the cloaca at some radial position and as a result there will be formed so-called coelo-anal canals in such a manner as mentioned above. The interradianal area of the cloacal wall is not broken even when it is influenced by such pressure as above mentioned from the reason that this area is strong enough being attached by the radial cloacal muscles connecting the cloacal wall with the body wall. In ordinary cases the so-called coelo-anal canal thus formed will be closed first by the coagulation of the blood and then it will gradually disappear in advance with the regeneration of the cloacal wall.

I did not find the so-called coelo-anal canal in the case of *Molpadia roretzii* (Table II) but in the case of other species of *Caudina*, already mentioned, I was able to find the same structure (fig. 1, *cc*).

We occasionally observe that the captured *Caudina* discharges its blood from the posterior end of the tail. In this case the blood is that discharged from the coelom passing through either the so-called coelo-anal canals or the five pores formed at its posterior end by some damage added to the tail end and the amount of blood discharged from the so-called coelo-anal canals should be rather small compared with that from the five pores above mentioned.

In *Caudina chilensis* we often meet with individuals with their so-called coelo-anal canals not yet completely formed but it is rather rare to meet with individuals provided with these canals completed (Table I). At any rate it seems to be highly probable that the so-called coelo-anal is a lesion caused by some pressure originated from the body fluid and is not the result of artificial injury.

SUMMARY

1. The circular anal cavity exists in the anal region of *Caudina chilensis*, *Molpadia roretzii*, and a species belonging to the genus *Caudina* which specific name is not determinable. This cavity seems to have some relation with the movement performed by the anus in the cases of these Holothurians

2. The so-called coelo-anal canal is formed occasionally in the case of *Caudina chilensis* of over 30 mm. body length. It seems to be a lesion formed by some pressure which occurred in the body fluid of the Holothurian.

3. In *Caudina chilensis* the blood is often discharged from the coelom through five pores which were formed by the damage given to the tail end but sometimes it is discharged also through the so-called coelo-anal canals.

LITERATURE CITED

- CLARK, H. L. 1907 The Apodous Holothurians. A Monograph of the Synaptidae and Molpadiidae.
- DENDY, A. 1898 On some points in the Anatomy of *Caudina coriacea* HUTTON Journ. Linn Soc. London, Zool. Vol. 29, No. 3, p. 456.
- GEROULD, J. H. 1896. Anatomy and Histology of *Caudina arenata* GOULD. Bu'l. Mus. Comp. Zool. Vol. 29, p. 121.
- HEDING, S. 1931. On the classification of the Molpadiids Vid. Medd Dansk. Naturh. Foren., Bd. 92, p. 275.
- . 1932. *Paracaudina* nom. nov. op. cit. p. 455.
- . 1933 The *Caudina* of Asamushi, The So-Called *Caudina chilensis* (J. MÜLLER). Sci. Rep. Tōhoku Imp. Univ. Biol., Vol. VII, No. 2, p. 127.
- HŌZAWA, S. 1928. On the Changes occurring with Advancing Age in Calcareous Deposits of *Caudina chilensis* (J. MÜLLER). do. Vol. III, No. 3, p. 361.
- INABA, D. 1930. Notes on the Development of a Holothurian, *Caudina chilensis* (J. MÜLLER). do. Vol. V, No. 2, p. 215.
- KAWAMOTO, N. 1927. The Anatomy of *Caudina chilensis* (J. MÜLLER) with Especial Reference to the Perivisceral Cavity, the Blood and the Water Vascular Systems in their Relation to the Blood Circulation. do. Vol. II, No. 3, p. 239.
- KITAO, Y. 1933. Notes on the Anatomy of the Young of *Caudina chilensis* (J. MÜLLER). do. Vol. VIII, No. 1, p. 43.
- KOIZUMI, T. 1932. Studies on the Exchange and the Equilibrium of Water and Electrolytes in Holothurian, *Caudina chilensis* (J. MÜLLER). do. Vol. VII, No. 2, p. 259.
- LUDWIG, H. 1899-92. Echinodermen. I. Die Seewalzen. In Bronn's Klassen und Ordnungen des Thier-Reichs. Bd. II.
- MITUKURI, K. 1912. Studies on Actinopodous Holothurioidea. Journ. Coll. Sci. Imp. Univ. Tōkyō. Vol. XXIX, Art. 2.

STUDY OF *EURYALE FEROX* SALISB.

VIII. MISCELLANY

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(With Plate XII and one text-figure)

(Received November 22, 1934)

A) MORE ABOUT THE DELAYED GERMINATION AND THE LONGEVITY OF THE SEED

For several years, the author has been engaged in the study of *Euryale ferox* SALISB., and has already reported about the delayed germination of the seed¹⁾. It has been established so far, along with other facts, that the seeds of *Euryale* ripen in the autumn towards October in this country, that they cannot germinate in the same period, that the germination percent is either nil or extremely low, if any, in the first spring directly following the harvest, that the majority of the seeds sprout in the second spring (i. e. after the lapse of about eighteen to twenty months from the time of the harvest), and that those then remaining dormant continue to be highly refractory to germinative conditions. In the course of time, some of these dormant seeds become infected by microbes and disintegrate. The majority of them are, however, quite resistant and their potential of germination remains uninjured for several years, which fact is easily conceivable from the sporadic germination of these stubborn seeds.

Now, the question naturally arises how long they can keep the viability under the ordinary condition of stratification. Concerning this point, exact records available are not very numerous so far²⁾. So that it was planned, in 1927, to keep a record of the germination of the *Euryale* seeds brought to the laboratory. In the summer of 1931, the author went abroad and was absent until the beginning of 1934, and the study was accordingly discontinued during this period, regardless of his eager desire to keep

¹⁾ OKADA, Y. 1925. On the germination of *Euryale ferox* SALISB. Bot. Mag., Tokyo, vol. 39, pp. 133-141. Do 1930. Study of *Euryale ferox* SALISB. V. Sci. Rep., Tōhoku Imp. Univ., 4 ser, vol. 5, pp. 41-116.

²⁾ For instance, the present author had occasion to observe the case of a dormant period of about three and a half years with an *Euryale* seed stratified in mud saturated with water.

on recording uninterruptedly. It was only after his return to Sendai that the seeds were again brought under observation. Towards the beginning of the summer of 1934, it was noticed that some of the seeds remaining dormant through these few years, began to mobilize, of which the records are given below.

Lot number of the sample	Number of seeds	Date of collection	Number of seeds germinated in						
			1928	'29	'30	'31	'32	'33	'34
No. 715	10	17/X/1927	6 ¹⁾	0	0	?	?	?	1
No. 8052	10	12/X/1928		0	2	?	?	?	1
No. 8054	10	12/X/1928		0	5	?	?	1	3
No. 800	60	12/X/1928		0	8	?	?	?	3
No. TK 863	20	12/X/1928		0	3	?	?	?	1
No. 900 A ²⁾	ca 10,000	15/X/1929			4	?	?	?	141
No. 900 B ³⁾	ca. 7,300	15/X/1929			21	?	?	?	103

Seeds of Nos. 715, 8052 and 8054 were kept under water at room temperature. Those of the other lots were stratified in mud saturated with water. The containers for Nos. 800 and TK 863 were placed inside of a window facing south, and Nos. 900 A and 900 B were kept in an 'Osakamuro' (a kind of covered space, not artificially heated).

¹⁾ Germination due to the forcing effect of temporary refrigeration, an exceptional case. ²⁾ Seeds of larger size, more than 10 mm wide across. ³⁾ Seeds of smaller size, less than 10 mm wide across.

It is much to be regretted that the history of these lots of seeds for the period from 1931 to 1933 remains unknown. However, it is established at least that the seeds of *Euryale* keep their germinability for a period not less than six years and a half if they are properly stratified under water.

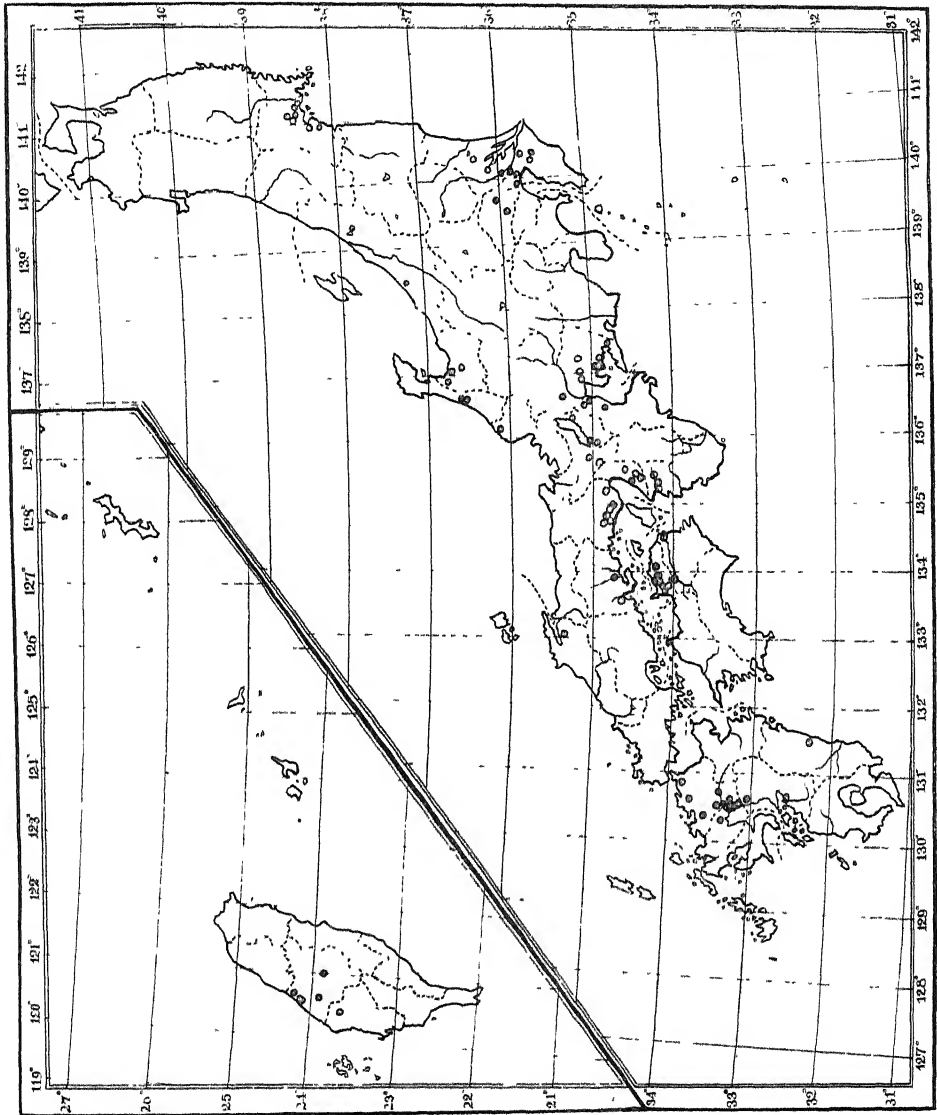
B) DISTRIBUTION IN JAPAN

In the geological period, the genus *Euryale* was presumably growing in Europe as well, although completely extinct there at present. Fossils of plants closely related to the living species so far have been discovered in several localities in Europe, e.g. *Euryale limburgensis* from pliocene stratum of Tegelen¹⁾, and *Euryale europaea* from interglacial deposit at Likhvin in Kaluga²⁾. In the present age, however, their natural habitat

¹⁾ Encycl. Brit., 1911. 11 Ed. vol. 20, p. 555.

²⁾ WEBER, C. A. 1907 *Euryale europaea*, nov. sp. foss. Ber. Deut. Bot. Ges., vol. 25, pp. 150-157.

is limited to southern Asia, and especially to the tropical and subtropical regions. They are found growing wild from India in the south to as far



Distribution of *Euryale* in Japan.

represents the locality where the plant is found to grow wild.

as Manchuria in the north¹⁾.

In our country, the range of their distribution covers the islands of Taiwan (Formosa), Kyûsyû, Shikoku and Honsyû. In Hokkaidô we have not yet any locality reported. The northern limit of the distribution seems to correspond to about 38° 30' N on the Pacific coast and 37° 55' N on the Japan Sea coast.

In order to have a bird's-eye-view of the geographical distribution in Japan, the author tried to plot all of the localities so far in his knowledge, on a chart which is inserted here. This is not intended to represent in any way the area occupied by the plant, nor the density of the vegetation, as exhaustive information concerning them is not available in the present circumstance. As for the names of these localities, the author considers it more appropriate to give them in detail elsewhere in Japanese, for it is a matter of but local interest.

C) RECORD OF A GIGANTIC LEAF

Euryale ferox is well known as the plant bearing the largest leaf in our country. Naturally the species is subject to radical variations, some producing leaves of remarkable size, while others produce those of minor dimension. There is among them one especial species growing wild in Toyama Prefecture that may be called almost gigantic (not only are the leaves gigantic, but so also are other organs of the plants of this race, and some statistical studies were formerly reported about this race as compared with plants of other localities)²⁾.

In 1928, the author measured in Zyûnityôgata, Toyama Prefecture, a leaf 1.91 meters wide, which was reported in this journal with photographs³⁾. In 1933, the growth of *Euryale* in the said locality was exceedingly luxuriant and some leaves surpassed 2 meters in diameter. The largest one which measured 2.42 meters (=8 'syaku' in Japanese unit) wide was photographed by Mr. MATOBA, formerly village-master there. This example seems to be one of the largest leaves ever recorded in our country, and may even rival *Victoria regia* in the dimension of the leaf surface. The photograph inserted here is a reproduction from the original by Mr.

¹⁾ REGEL, E. 1862. Tentamen Florae Ussuriensis. Mém Acad. St. Pétersb. 7 sér., tome 4, n° 4, pp. 15-16. KOMAROV, V. L. 1901-07. Flora Manchuriae (Transl. Jap. vol. 3, fasc. 2, p. 4, 1927-33).

²⁾ OKADA, Y. 1928. Study of *Euryale ferox* SALISB. I, II. Sci. Rep., Tôhoku Imp. Univ., 4 ser., vol. 3, p. 271, p. 581.

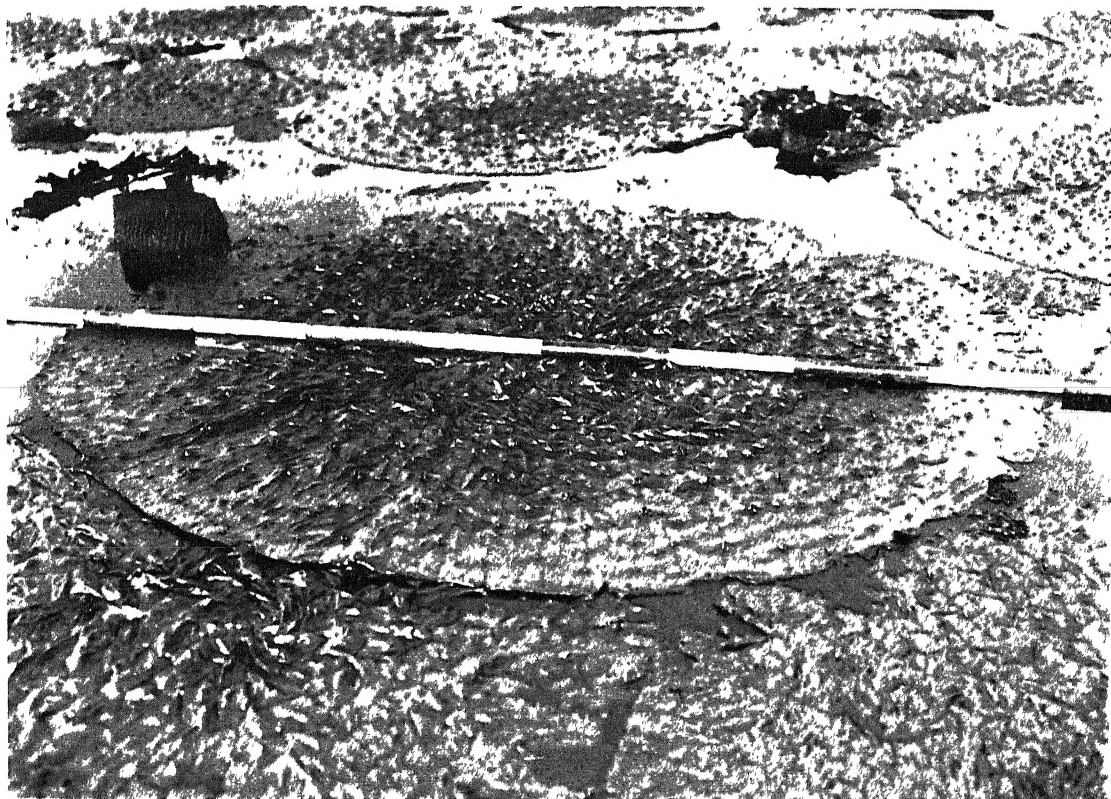
³⁾ OKADA, Y. 1929. Study of *Euryale ferox* SALISB. IV. Ibid. vol. 4, p. 361.

MATOKA for whose kind permission the author wishes to express his cordial acknowledgement.

EXPLANATION OF PLATE XII.

Gigantic leaf of *Euryale ferox* SALISB

Overlying on the leaf surface is a measuring pole which serves to show the dimension of the leaf. One division of the pole corresponds to one 'syaku' or 0.303 meters. Aug. 27, 1933. H. MATOKA photo



Y. OKADA: Study of *Euryale ferox*. VIII.

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